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EFFECTS OF TEMPERATURE AND SALINITY ON GROWTH,  
FOOD CONVERSION, SURVIVAL AND TEMPERATURE  
RESISTANCE OF JUVENILE BLUE CRABS,  
*Callinectes sapidus* RATHBUN

Prepared by

J. S. HOLLAND, D. V. ALDRICH and KIRK STRAWN

Department of Wildlife and Fisheries Sciences  
Texas A&M University

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CONVERSION, SURVIVAL AND TEMPERATURE RESISTANCE OF  
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## ABSTRACT

Effects of Temperature and Salinity on Growth, Food Conversion,  
Survival and Temperature Resistance of Juvenile Blue Crabs,  
Callinectes sapidus Rathbun. (August 1971)

Caged juvenile blue crabs, Callinectes sapidus Rathbun, (5-40 mm) were maintained at different temperatures (15-35 C) and salinities (1-21‰) for 30-45 days. Underwater weight was recorded for individual crabs at 5-day intervals. Growth, yield and food conversion efficiencies were calculated. Mortalities and molts were recorded daily for caged crabs. All crabs were fed a pelleted artificial diet.

Uncaged juvenile blue crabs were maintained for 25 days at combinations of three temperatures (20, 25 and 30 C) and three substrates (glass, sand, and sand-plus-shell). Survival for uncaged crabs was recorded after 15 and 25 days. Weights of initial groups (N=50) of uncaged crabs and of survivors after 15 and 25 days were recorded.

Survivors from the various experiments were subjected to lethal temperatures (36-42 C). Survival time, sex and weight were recorded for crabs in lethal temperature tests.

Growth and food conversion were closely related to temperature. The optimum temperature was 29-30 C. Yield (weight gain) and survival were also temperature related but were affected differently for caged and uncaged crabs.

Yield of caged juvenile blue crabs was indirectly related to mortality. Mortality was directly related to temperature above 30 C. Yield of uncaged blue crabs was directly related to mortality, which varied directly with temperature (20-30 C). The latter effect was due to cannibalism. Substrate affected cannibalism among uncaged juvenile blue crabs. A sand-plus-oyster shell combination provided better survival than either sand or glass substrate. There appeared to be no difference between sand and glass substrates in preventing cannibalism.

Very low salinity (1‰) was apparently lethal to small blue crabs at optimal growth temperatures (29-30 C). Salinities above 1‰ had no significant effect on growth, yield, food conversion or survival.

The upper incipient lethal temperature of juvenile blue crabs appeared to be 33 C, based on a 45-day cage study, attributing all

deaths in that study to temperature stress. One thousand minute  $TL_m$  for crabs acclimated to 20, 25 and 30 C were 37.1, 38.6 and 39.4 C, respectively.

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## INTRODUCTION

The blue crab, Callinectes sapidus Rathbun, is primarily an estuarine organism. Thus, it is subject to man's depredations of estuaries and their biota. Man's influence upon many estuarine areas of the world has altered this environment to the extent that many natural, commercially important populations have been decimated or reduced to a level at which they are no longer valuable to man (Korringa, 1967).

Rees (1967) ranks the blue crab as economically the third most important marine organism of Chesapeake Bay, the South Atlantic coast and the Gulf of Mexico, outranked only by the shrimp, Penaeus sp., and oyster, Crassostrea virginica. Although large populations of blue crabs exist along the Atlantic and Gulf coasts of the United States, annual fluctuations in abundance create many problems for the fishing and packing industries dependent upon these populations (Pearson, 1948; Tagatz, 1965; More, 1969). Rees (1962) and More (1969) have shown that knowledge to control or predict the fluctuations is not available. The effects of many man-made changes in estuarine environment upon natural populations of blue crabs cannot

be predicted at the present time but destruction of the estuarine environment would certainly lead to the destruction of natural blue crab populations.

Thermal loading of estuarine waters by industrial sources, particularly electrical power generating plants, is of growing concern (Cronin, 1967; Mihursky and Kennedy, 1967; Pritchard and Carter, 1965). Some investigations (Costlow, 1967a; Cheung, 1969; Darnell, 1969; Daugherty, 1952) indicate that spawning, hatching and larval growth of the blue crab and other allied species are temperature dependent and are successful only within certain temperature ranges.

A possible solution to the fluctuation of abundance of natural populations of blue crabs and a means of alleviating the thermal loading of estuaries lies in pond culture. By heating culture ponds with thermally-loaded water, two beneficial results could be realized. First, blue crabs could be kept at temperatures at which they could grow during periods when natural water temperatures would prevent their growth. Second, culture ponds could serve as retention basins to lower the temperature of heated water before it is returned to the estuary.

## OBJECTIVES

The general objective of this study is to supply information vital to the commercial culture of the blue crab on the Texas Gulf Coast.

Specific objectives include:

1. To ascertain optimum levels of water temperature and salinity for the growth of juvenile blue crabs.
2. To determine the effects of temperature, salinity and substrate on the cannibalism and mortality of juvenile blue crabs.
3. To study food-conversion rates at different temperatures, salinities and feeding rates.
4. To investigate the thermal tolerance of juvenile blue crabs acclimated at various temperatures and salinities.

## PRESENT STATUS

The blue crab, due to its wide range and economic importance, has been the subject of much scientific research and subsequent literature. Its life history has been clearly established by Hay (1905) and Churchill (1919). More detailed descriptions of the larval stages are given by Hopkins (1943, 1944), Churchill (1942) and Sandoz and Hopkins (1944). Life histories for the blue crab in various parts of its range are given by Van Engel (1958) and Pearson (1948) for Chesapeake Bay, Daugherty (1952) and More (1969) for Texas, Darnell (1959) for Louisiana, Tagatz (1968a) for Florida, and Williams (1965) for the Carolinas.

A large part of the research on blue crabs is fishery-oriented. Of particular interest in fishery-oriented blue crab research are: (1) population fluctuations (More, 1969; Pearson, 1948; Fischler, 1965; Tagatz, 1965); (2) adult migrations (Tagatz, 1968a; Fischler and Walburg, 1962; Daugherty, 1952; More, 1969); (3) catch-effort, catch-composition and gear studies (More, 1969; Pearson, 1948; Van Engel, 1962; Cummins and Rivers, 1962; Fischler, 1965); (4) larval and juvenile abundance and distribution studies (Rees, 1962, 1967; Pearson, 1948; More, 1969); (5) pesticide, disease and

parasite mortality studies (Sprague and Beckett, 1966; Hopkins, 1947; Rawls, 1965; Sprague, 1966; Lowe, 1965; Beaven and Truitt, 1940; Reinhard, 1950; Rogers-Talbot, 1948; Sandoz, Rogers and Newcombe, 1944); and (6) food technology (Bernarde, 1961; Farragut, 1965).

Other research pertinent to the commercial culture of the blue crab is found in the larval crab studies of Costlow (1963a, b, 1965, 1966, 1967), Costlow and Bookhout (1959, 1966a, b, 1967) and Costlow, Bookhout and Monroe (1966). These studies demonstrate the feasibility of raising blue crabs from the egg to the first crab stage, an important prerequisite in the commercial culture of crabs. These investigators also described the effects of temperature and salinity on the larval forms of blue crabs and other crab species.

Environmental effects upon thermal tolerance of juvenile and adult blue crabs have not been adequately researched, although Tagatz (1968b, 1969) has shown some basic relationships between temperature, salinity and thermal tolerance in adult female and large immature blue crabs. No paper has been published concerning the temperature tolerance of small (5-40 mm) blue crabs.

## GENERAL PROCEDURES

This study was set up as a series of integrated experiments, designed to achieve the objectives as previously stated. Preliminary experiments, using small numbers of crabs to become acquainted with collection, care and handling of these organisms were thought to be necessary. The preliminary experiments were also used to establish lethal test techniques and to obtain basic information on the thermal tolerance of juvenile blue crabs.

A study of the acclimation time of juvenile blue crabs to a high temperature (35 C) was needed early in the experiment to establish minimum acclimation time. This time was necessary to prevent the use of non-acclimated crabs in lethal resistance studies.

An individual death time study was conducted to obtain information on the survival of crabs subjected to various lethal temperatures. This information was used to decide upon lethal temperatures that would cause deaths within certain time ranges, and was useful in planning further lethal tests. This study also gave basic information as to the probability of different physiological death mechanisms affecting juvenile blue crabs.

Three different studies on the growth, mortality, yield and food conversion rate of blue crabs were made. Some of these crabs were used in lethal tests upon termination of the growth studies.

The first growth study defined the effects of a range of relatively high temperatures upon the mortality, growth, yield, and food conversion efficiency of juvenile blue crabs. The optimum temperature in this study was used in further growth studies.

A second study established the effects of salinity upon small blue crabs. A range of salinities (1-21‰) which might be found in estuaries was used.

Due to low survival rates at low salinities in the preceding growth test, the third growth study investigated the effects of optimal and low temperature on growth and mortality at very low salinities, to find a minimum salinity level for juvenile blue crabs.

Survival at lethal temperatures was tested to ascertain the effects of various acclimation temperatures and salinities on survival times. Due to high mortality that occurred during the salinity-growth study, little salinity data was obtained. The effects of various acclimation temperatures ranging from 20-35 C upon survival at lethal temperatures (36-42 C) were tested.

Mortality due to cannibalism was thought to be a major cause of death in populations of blue crabs. A mortality-substrate study was designed to study the effects of various substrates upon cannibalism. The survivors from this study were used in lethal test experiments also.

Other data were to be gleaned from all of the previous studies. These data would include length-weight ratios, relationships of underwater, wet-in-air, and oven-dried weights of individual crabs, effects of sex, weight and width on survival times, and data on the molting of juvenile blue crabs.

Small crabs (5-35 mm carapace width) were collected from Galveston Bay. Collections were made off the screens at the P.H. Robinson Generating Station in Bacliff, Texas, and by seines, bar-trawls and push-nets. The most successful method of collecting the juvenile crabs was by using a push-net (Strawn, 1954) through grassy or slightly muddy areas of shallow water.

Acclimation and growth studies were conducted in 80-gallon aquaria (Figure 1) constructed of plywood laminated with polyurethane foam for insulation and coated with a polyester resin (Laminac 4110). The aquaria were designed so that temperature, salinity, and light could be controlled with precision. Each aquarium was completely enclosed and provided with a light-proof door for easy accessibility. Temperature was controlled through a relay system consisting of a contact thermometer (Jumo MS DBP 2.69) which, by making or breaking an electrical circuit through the movement of the mercury column, caused a relay to turn on or off a submersible electric heater. Ten aquaria were constructed with direct



Figure 1. Interior of acclimation tank with acclimation cages suspended above water line. Cages are approximately 7.5 cm wide.

refrigeration systems. In these, the heating and cooling systems were antagonistic. Temperature control in the acclimation tanks was  $\pm 0.1$  C between 0-50 C in the directly-refrigerated tanks and from ambient to 50 C in the uncooled tanks.

Light in each of the enclosed acclimation aquaria was provided by a single 30-watt fluorescent bulb mounted within a wooden and glass box. It was suspended from the ceiling of the tank so that the lamp was approximately 10 inches above the water surface. All metal parts of the light fixture were mounted outside the aquarium to prevent corrosion of the fixture and metal ion contamination of the aquaria. Photoperiod was controlled by electrical timers (Tork Time Controls). All experiments were conducted with 12 hours of light per day. Lights were turned on at 6 AM and off at 6 PM daily. The doors remained closed unless work was being done within the aquarium.

Salinity within each acclimation aquaria was checked daily, using an American Optical Total Solids Meter. Salinities were set originally by mixing seawater and Galveston city water in proper portions. The water was allowed to set several days before introducing crabs into the aquaria. Achieving various salinities used was facilitated by the fact that 1 inch of water in the acclimation aquaria was equivalent to 5 gallons. By knowing the salinity of the

seawater available, the salinity of the tap water (about 0.5‰ in Galveston) and the volume of water needed, a very close approximation of the volumes of seawater and tap water required to give the total volume of water at a given salinity was calculated by the equation:  $(V-X) S + XT = VF$ , where V is the final volume required, S is the salinity of the seawater, X is the volume of tap water, T is the salinity of the tap water, and F is the salinity of the water required. Once the correct volume of water at the proper salinity had been established, a mark was made at the water-line to facilitate maintaining the salinity. As the level dropped through evaporation, it was readjusted to the original volume by addition of distilled water. Salinity was verified with a salinity meter. Salinity fluctuated less than 1‰ throughout the study due to the frequent adjustments and low evaporation in the enclosed tanks.

Seawater was acquired from Sea Arama, a commercial marine aquarium located on Galveston Island. It was pumped from beyond the surf zone of the Gulf of Mexico and transported by personnel and equipment of the National Marine Fisheries Service Laboratory of Galveston, Texas, to a concrete holding tank outside our laboratory, from which it was gravity fed into the laboratory.

Each acclimation aquarium was supplied with compressed air from four airstones and two substrate filter mechanisms. This

arrangement provided sufficient oxygen for crabs kept in aquaria and provided sufficient water circulation to maintain the desired homogeneity of water temperature.

Twenty-two lethal test aquaria were constructed for use in thermal acclimation studies (Figure 2). Each was an 8-gallon aquarium constructed of plywood and plexiglass. The plywood was sealed with a polyester resin (Laminac 4110). The same type of temperature control equipment was used as in the acclimation aquaria, again holding temperature within  $\pm 0.1$  C. Compressed air from two airstones provided oxygen and water circulation for temperature homogeneity. No filtration was used in the lethal test aquaria as the water was changed prior to each series of lethal experiments. No feeding was done in the lethal test tanks. Each tank was equipped with an elapsed-time meter which was calibrated in tenths of a minute.

Crabs in the growth and acclimation experiments were kept at all times in individually numbered cages to prevent mortality from cannibalism and to allow study of individual crabs. The cages were of two types. Those used in the growth studies (Figure 3) were made of plastic mesh and plexiglass. Each was approximately 9 x 7.6 x 5 cm. The cages (Figure 4) used in the lethal tests were smaller, being approximately a 5 cm cube of plastic mesh with one plexiglass side for observation.

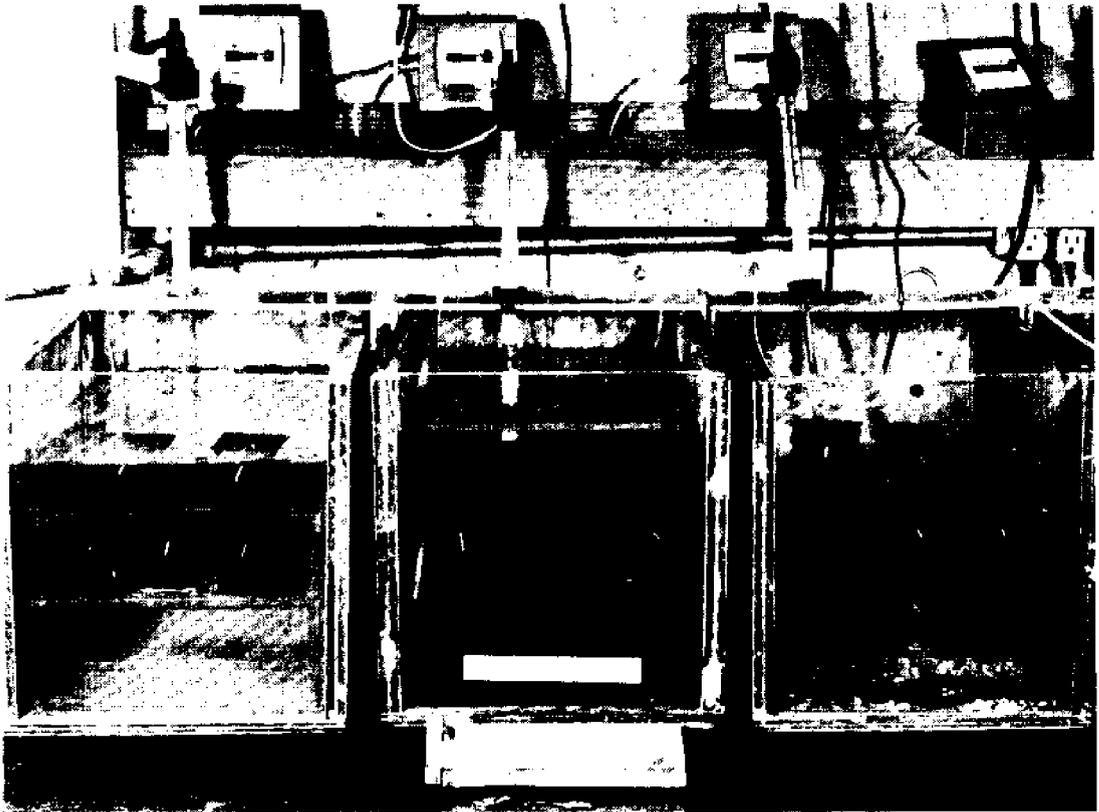


Figure 2. Lethal test tanks with control equipment and timers. Lethal test cages are shown on plexiglas racks. Ruler on front of center tank is 15 cm long.

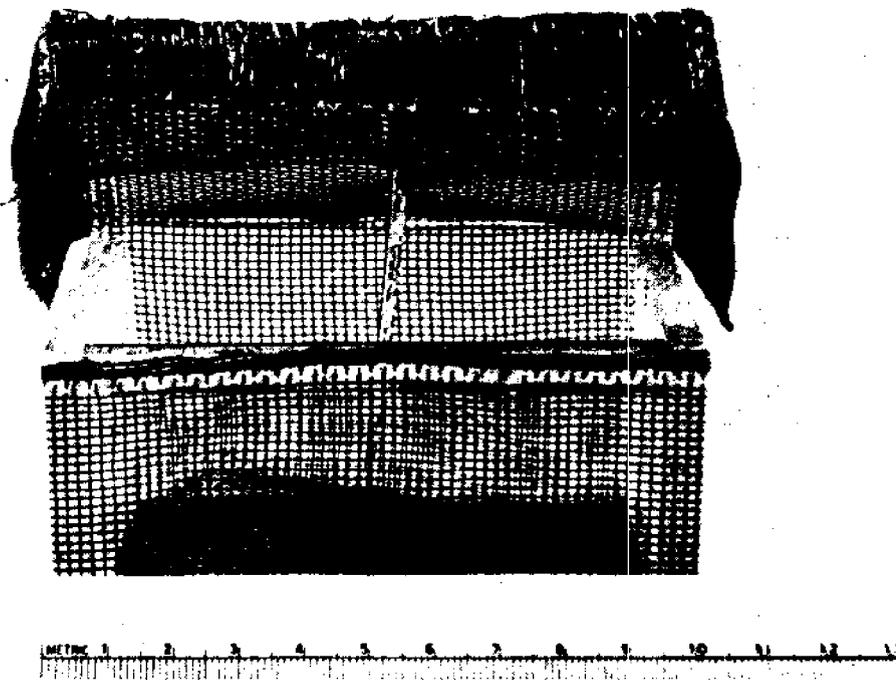


Figure 3. Acclimation cage with top open. Slightly smaller than natural size.

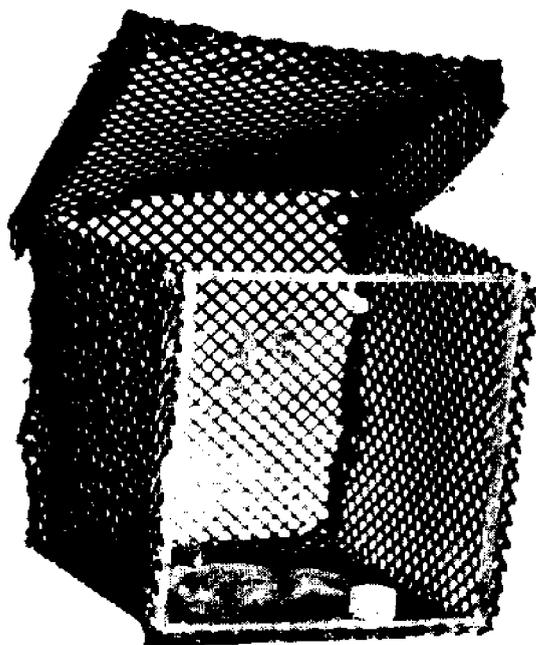


Figure 4. Lethal test cage with crab and food pellet.

Slightly smaller than natural size.

Lethal tests were formed by introducing samples of crabs directly into the lethal baths which had been stabilized at the test temperature and salinity. Each lethal test consisted of a maximum sample of five individually caged crabs. At high lethal temperatures (40-43 C), the lethal tests were conducted upon individual crabs due to the difficulty in trying to keep accurate records when crabs in a larger sample were all dying simultaneously. After introduction into the test conditions, the crabs were watched constantly until death determinations were made. Death was normally preceded by total loss of movement of the appendages. When this stage was reached, the crabs were manipulated in their cages so that movement of the gill-bailer was the ultimate criterion for death of crabs in this study. All deaths were recorded to the nearest 0.1 minute. I feel that these recordings are accurate to  $\pm 0.1$  minute for survival times of less than 100 minutes and 0.5 minute in longer survival times. Death times of small, translucent crabs were easier to determine than those of large, opaque specimens. Following death, the crabs were sexed, measured for carapace width to the nearest millimeter and weighed wet.

Three types of weight measurements were taken. All live weights of individuals were made underwater. Small, variable amounts of water caused the in-air weights of the juvenile blue crabs to be very

unreliable. Havinga (1928) and Andrews (1963) found that underwater weights of oysters were very accurate and repeatable. Using this method for small blue crabs provided accurate ( $\pm 2$  mg) measures of growth of small blue crabs with very good repeatability. For these, a Mettler H23 balance was set up over a waterbath (Figure 5) maintained at a constant temperature and salinity. A plastic mesh envelope (Figure 5) was developed which would hold the crabs and release them easily, but restricted movement of the crab which would have disturbed the balance reading. Several of these envelopes were made to weigh the same amount to the nearest milligram,  $\pm 2$  mg. At each weighing period, the balance was zeroed with each of the weighing envelopes on a plexiglass plate suspended in the waterbath. Ten crabs were taken from the acclimation aquaria, each in its numbered cage. Each crab was removed from its cage, placed in a weighing envelope, weighed and replaced in its cage. The empty envelopes were weighed periodically to make sure the balance remained properly zeroed. Movement of the crab-containing envelope in and out of the water was done very gently to prevent a seiche that affected the balance readings. The submersed heater caused convection currents which affected accuracy of the balance. Water temperature was maintained by periodically turning on the control and then waiting until convection currents subsided. Changing water

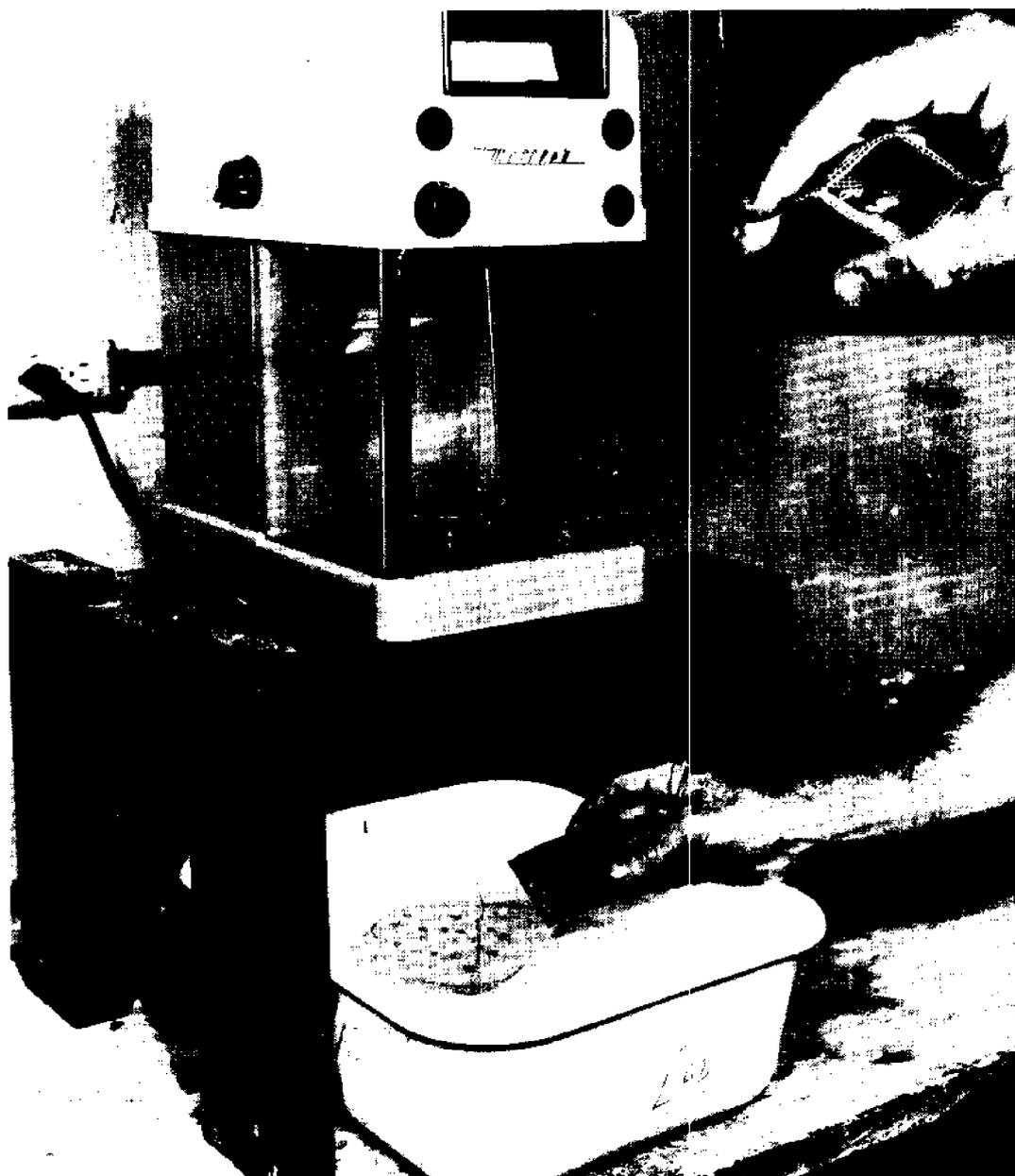


Figure 5. Underwater weighing set-up. Inset shows crab in the opened weighing envelope.

in the balance waterbath had to be done at least 6 hours prior to weighing and then only if the water was aerated minimally. If not, air bubbles tended to come out of solution and adhere to the plexi-glass plate. This changed the zero point of the balance. Care also was taken in placing the mesh envelopes in the water to prevent trapping an air bubble. By keeping the envelopes wet at all times between weighing periods, this possibility was diminished.

Wet weights were made on dead crabs from the lethal tests and on samples of 50 live crabs from the substrate study. These measurements were made on a Sartorius 2254 balance and reported to the nearest 0.01 gm. The crabs were allowed to drain on absorbent paper toweling before weighing.

Dry weights were made on selected crabs from the substrate study. The crabs had been weighed by the two previous methods. They were placed in a drying oven at approximately 100 C and dried for 24 hours. Weights to the nearest milligram were then made on a Mettler P160N.

All crabs were fed a pelletized mixture of 50% fish flour and 50% Milk Nutrient Concentrated (MNC). Pellets were formed by pressing the mixture through a pellet press. Samples were weighed from each batch of pellets. All the pellets within a batch were very uniform but there was slight variation in pellet sizes between batches. The

pellet press used was property of the National Marine Fisheries Laboratory in Galveston. Personnel of that laboratory made pellets of different sizes. It was impractical to re-set the press to try to make exactly (nearest milligram) the same size pellets between batches.

Crabs were fed once or twice a day. They were fed 1-4 pellets per day according to the experiment. Each crab in an experiment was fed the same amount as every other crab, independent of size or weight.

Dead crabs were replaced upon discovery. The weight of the sample of crabs kept in each acclimation tank was corrected so that only weight incurred under test conditions was used in subsequent calculations.

## SPECIFIC PROCEDURES

### Preliminary Study

Crabs for this study were collected from the screens at P. H. Robinson Generating Station on September 31, 1969. They were held for 48 hours at 35 C prior to the tests. Salinities of 10 and 16‰ were used in both acclimation and lethal baths. Thirteen small crabs were subjected to lethal tests of temperatures from 40.5 to 43 C. These crabs were measured for carapace width and sexed.

### Acclimation Rate Study

Crabs were collected October 2, 1969, off the screens at P. H. Robinson Generating Station in Bacliff, Texas. Water temperature was 27.1 C and salinity was 20.5‰. The crabs were held overnight in an unheated holding tank, 26 C and 10‰. On October 3, 1969, 49 crabs were placed in cages in acclimation aquaria at 35 C and 10‰. A preliminary sample of nine crabs, presumably acclimated to field conditions, were subjected to a lethal temperature (42 C). Seven lethal tests, the first starting 3 hours after introduction into the 35 C water with the time doubling between tests, were planned. Sample size was to be seven crabs. Several of the latter tests were completed upon smaller samples due to the discovery of a second species of crab

(*C. similis*) in the acclimating samples. Crabs were fed one feed pellet daily (200 mg), 24 hours prior to the time they were to be subjected to the lethal tests. The last lethal test was after 8 days of acclimation.

#### Individual Death Time Study

Crabs were collected on November 7, 1969, from P. H. Robinson Generating Station at Bacliff, Texas. They were caged individually and held for 6 days at 35 C and 15‰ salinity. Two samples of five crabs each were tested at each of the following lethal temperatures: 42, 41.5, 41.0, 40.5, 40.0 and 39.5 C. These crabs had not been fed for 24 hours prior to the lethal test. A 13th sample was fed and introduced 25 minutes after feeding. Carapace width and sex were recorded for each crab.

#### Temperature-Growth Study

Crabs for this study were collected from West Bay, near San Luis Pass, on November 22, 1969. This study was originally designed to study the growth of crabs at various temperatures and to examine the possibility of using oysters as biological filters. Oysters were known to be susceptible to Dermocystidium marinum at elevated temperatures. Very small oysters, less susceptible to the fungal disease, were used

in this experiment. The ability to use oysters as biological filters would have indicated possible polyculture of oysters and crabs. Nine temperatures, ranging from 27 to 35 C in 1 degree intervals were used. At 27, 29, 31, 33 and 35 C, replicate aquaria containing 10 caged crabs and 10 numbered oysters were set up. At 28, 30, 32 and 34 C, pairs of aquaria were used in which one of each pair held 10 crabs and 10 oysters. The other held 10 oysters only. It soon became apparent that the oysters would not survive under experimental conditions due to D. marinum infections. The study was then continued with only the crabs.

The crab cages were placed directly upon the substrate in this experiment. It was found that uneaten food collected directly beneath the cages in the substrate material (sand and shell). Fungal growths were common on the substrate and were removed periodically. These growths were common at all temperatures although somewhat diminished at the two highest (34 and 35 C). Some cage bottoms became encrusted with fungus and were cleaned periodically.

Crabs were fed one feed pellet daily throughout the experiment. The size of the pellet was increased several times. Pellets fed during the first growth period weighed 200 mg, or approximately 66% of the initial weight of the individual crabs. Pellets used for the next six growth periods (30 days) had a mean weight of 302 mg (S.D. 21.6).

This represented 100% of the average initial weight of individual crabs. Pellets fed in the last two growth periods (10 days) had a mean weight of 325 mg (S.D. 39.2). This was about 108% of the average initial weight per crab and approximately 15% of the average final weight per crab. Each sample of 10 crabs was fed 135.5 grams of food during the 45-day experiment.

Crabs were weighed underwater every 5 days. Dead crabs were replaced daily. Molts were recorded daily.

Upon completion of the 45-day growth experiment, crabs were subjected to lethal temperature tests at 40 C and 15‰ salinity. Survival times, length, weight, and sex were recorded for each crab.

#### Salinity-Growth Study

This experiment was designed to ascertain the effects of salinity upon growth, mortality, and food conversion of small juvenile blue crabs. Five salinities, 1, 6, 11, 16 and 21‰ were replicated in pairs of acclimation aquaria. Temperature was maintained at 29 C. Crabs were collected from tidal ponds near the South Jetty, Galveston, Texas, on February 7, 1970. Water temperature in ponds was 17 C and salinity was 15‰.

Ten caged crabs were placed in each acclimation aquarium. Cages for this experiment were suspended above the bottom on a

plexiglass rack to facilitate cleaning of the aquaria and allow the uneaten food to disperse to the aquarium bottom. Only partial substrate filters were used in this and subsequent experiments. The uneaten food was siphoned periodically from the tanks to lessen the fungal growth in the tanks.

The number of food pellets was varied through the experiment. Pellet size (111 mg) was constant and smaller than used in previous experiments. One pellet was fed daily for the first 10 days, representing approximately 55% of the average initial weight of the small crabs. Two pellets were fed during the third growth period, three during the fourth growth period, four during the fifth growth period, and three during the final four periods. The crabs were thus fed from 55% to 220% of the average initial crab weight per day during the experiment. The final feeding rate (333 mg per day) was approximately 22% of the average final crab weight. Each sample of crabs was fed 127.65 gms of food during the 45-day study.

The crabs were weighed at 5-day intervals (growth periods). Dead crabs were replaced and molts were recorded daily.

#### Temperature-Salinity-Growth Study

Two temperatures, the proposed optimum from previous experiments (29 C) and one approximating a mean winter temperature for

Galveston Bay waters (15 C) were used in combination with four salinities: 1, 2, 4 and 6‰. Each temperature-salinity combination was replicated in a pair of acclimation aquaria, except those involving 1‰, which were triplicated due to the high rate of mortality at 1‰ in the previous experiment.

The samples of crabs were kept above the aquarium bottom as described previously. Fungal growths were removed daily.

The rate of feeding was unchanged throughout this experiment. Crabs were fed twice daily, one pellet (164 mg) per feeding. This amounted approximately 150% of the average initial crab weight per day. Each sample was fed 98.4 g of food in 30 days.

Dead crabs were replaced and molts were recorded daily. This study was completed in 30 days.

#### Mortality-Substrate Study

Three temperatures were studied in combination with three different substrates to find the effects of temperature and substrate on the mortality due to cannibalism in juvenile blue crabs. Three 7.5-gallon aquaria were placed in each 80-gallon acclimation tank which served as a constant temperature waterbath. Each of the three experimental aquaria contained a different substrate. One had only a glass bottom with no other substrate. The second had a 4 cm layer

of blasting sand. The third had a 4 cm layer of blasting sand plus 25 large oyster shell valves.

Each of the three temperatures used (20, 25 and 30 C) was replicated in a pair of acclimation tanks. The three 7.5-gallon aquaria were thus replicated at each temperature.

Eighteen samples of 50 crabs each were weighed underwater and wet-in-air on February 15, 1970. They were placed, uncaged, in the experimental aquaria. Twenty food pellets (111 mg each) were placed in each aquarium the first day. This represented 220% of the average initial sample weight. This amount was not eaten, so the number of pellets was decreased to 10 per day for the remainder of the experiment. On February 19, 1970, charcoal filters were installed in each experimental aquarium. On February 28, the surviving crabs were counted, weighed wet-in-air, and replaced in each aquarium. The experiment was terminated on March 10, 1970, when one of the samples was reduced to one crab. Survivor counts and underwater and wet-in-air weight determinations were made on each sample.

Upon termination of the substrate study, each of the survivors was subjected to lethal temperatures. The lethal temperatures varied because resistance to heat death is strongly influenced by acclimation temperatures. In order to get survival times from all three acclimation

temperatures within a suitable time range, lethal temperatures from 36 to 41 C were used.

Experimental aquarium, acclimation conditions, survival time, width, sex, underwater and wet-in-air weights were recorded for each crab.

Survivors from the substrate study that had lost no appendages and were otherwise normal were dried at 100 C for 24 hours and weighed. Comparisons of the three weights used in this study were made.

## OBSERVATIONS

### Preliminary Study

Preliminary tests on September 30, 1969, showed that juvenile blue crabs have a very high tolerance to heat. Crabs which had been collected from P. H. Robinson Generating Station in Bacliff, Texas, on Galveston Bay and placed directly in 35 C water showed no ill effects. After 48 hours of acclimation at 35 C, lethal tests produced survival times of 4-5 hours at 40.5 C (Table 1). Higher lethal test temperatures produced shorter survival times. No difference in survival times due to salinity differences was observed.

Crabs introduced into lethal baths of different temperatures in this and subsequent experiments showed various reactions. The intensity and sequence of certain reactions seemed dependent upon the temperature. Other reactions were common to all temperatures. Most crabs exhibited an initial inactive period which seemed to be temperature dependent. This initial reaction lasted longer at the lower temperatures in the lethal range (37-38 C). Immersion in extreme temperatures (42-43 C) seemed to produce a short or practically unnoticeable inactive period. In most cases, following the initial inactive phase came a period of violent activity. This period was short at the lower end of the lethal range and was followed

TABLE 1. --Survival time (at different temperatures and salinities), width and sex of juvenile blue crabs used in preliminary lethal tests.

Lethal Temperature	Salinity 10%			Salinity 16%		
	Survival time (minutes)	Width (cm)	Sex	Survival time (minutes)	Width (cm)	Sex
40.5	265.0	3.0	Female	300.0	1.9	Female
41.5	69.9	2.8	Female	128.0	4.1	Female
	46.4	3.5	Male	53.0	4.0	Female
	103.3	3.2	Female			
42.0	46.0	3.4	Female	49.0	3.2	Female
	60.0	4.2	Female	49.0	3.4	Female
43.0	13.5	3.6	Female	13.0	3.6	Female

successively by a long period of relatively normal activity, quiescence, then death. Mid-range lethal temperatures (39-40 C) produced a moderately long period of violent activity, no return to normal activity, a long period of quiescence and immobility, and eventual death. High lethal temperatures produced an immediate period of violent activity, no period of normal activity or quiescence. The violent period ended suddenly in death. A large percentage of crabs subjected to the high lethal temperatures in all lethal tests were extremely rigid upon removal from the lethal bath. This was not observed in any crabs subjected to the low or medium range of kill temperatures.

Loss of appendages (autotomy) was common at the high temperatures and occurred in some crabs at moderate temperatures.

A feature common to tests at all lethal temperatures was the emission of a viscous, slightly brown fluid from the mouth just prior to death. This was noted in the preliminary tests and most subsequent lethal tests.

#### Acclimation Rate Study

When a sample of nine crabs, acclimated to field conditions (27.1 C and 20.5‰ salinity) was subjected to lethal temperature of 42 C, the survival time for all crabs was less than 2 minutes.

Survival time for subsequent samples of seven crabs increased with acclimation time until it became steady between 98 and 192 hours of acclimation time (Figure 6). The last sample used was comprised of only two crabs.

#### Individual Death Time Study

After 6 days of acclimation at 35 C and 15‰ salinity, groups of juvenile crabs subjected to each of six lethal temperatures (39.5-42 C in 0.5 degree intervals) had survival time ranges of 10-70 minutes at 42 C and from 400-800 minutes at 39.5 C (Figure 7).

The first 10 samples of crabs used in this experiment had been fed 24 hours prior to the lethal tests. One sample of five crabs was fed and subjected to lethal temperature (42 C) 25 minutes after feeding. A two-way analysis of variance was completed on the logarithms of the survival times of crabs in the three samples subjected to 24 C (Table 2). The effect of feeding time upon survival time was significant. Multiple comparisons of means of survival times by the Least Significant Differences method and by the Studentized Range Q method indicated that one of the two samples fed 24 hours before the lethal test was significantly different from the sample fed 25 minutes prior to the lethal test, but the other was not. All subsequent tests were run 24 hours after feeding.

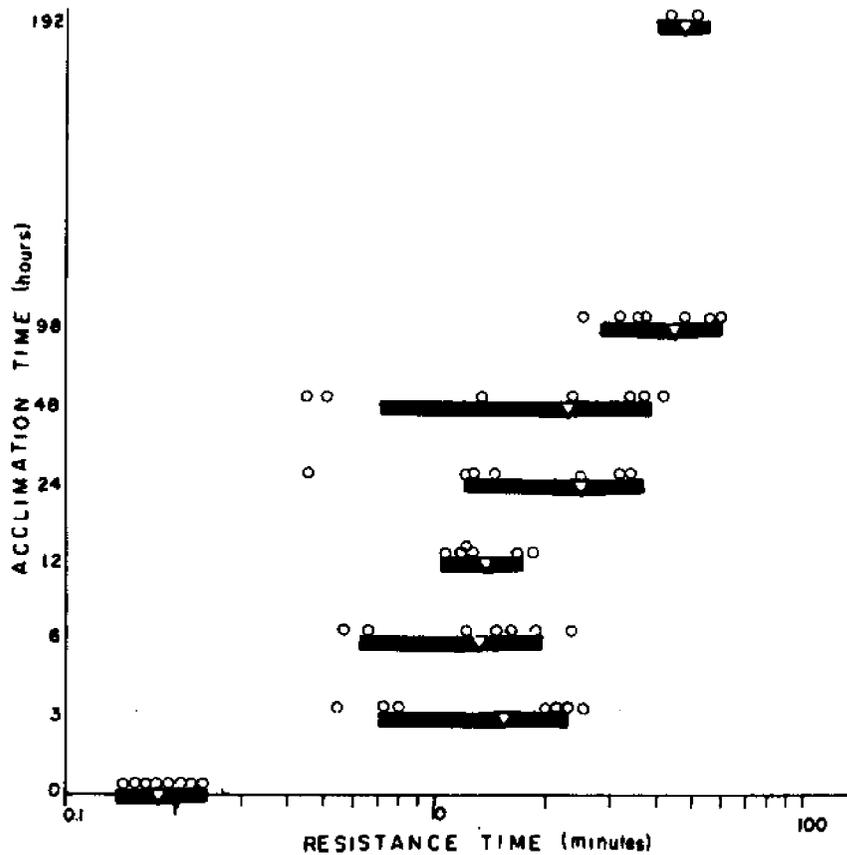


Figure 6. Acclimation rate of juvenile blue crabs to 35 C. Initial acclimation was to 27 C. Points indicated are mean survival times of samples subjected to 42 C. Black lines indicate one standard deviation about the mean. Circles indicate death times.

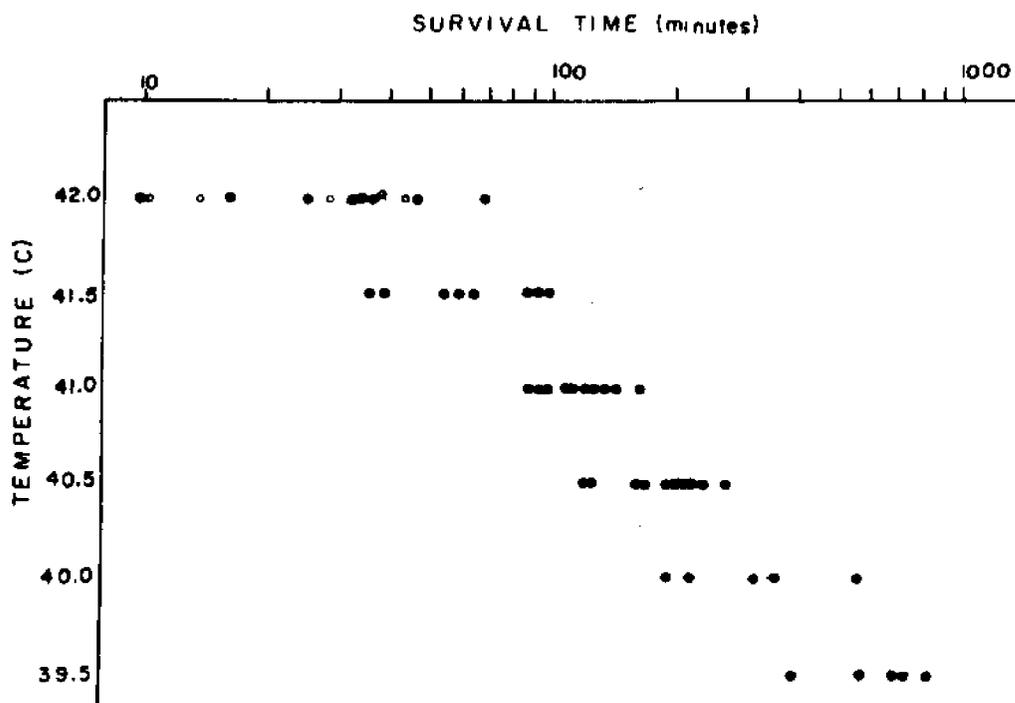


Figure 7. Survival of juvenile blue crabs acclimated 6 days to 35 C and subjected to various lethal temperatures. Circles indicate crabs fed 25 minutes prior to lethal test. Other crabs fed 24 hours prior to testing.

TABLE 2. --Randomized block analysis of variance of the logarithms of survival times of three samples of five crabs fed at different times prior to lethal tests.

Analysis of Variance				
Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	14	0.65782		
Feeding time	2	0.14849	0.07424	6.46689*
Blocks	4	0.41749	0.10437	9.03146
Error	8	0.09184	0.01148	

\*Significant ( $P < .05$ )

### Temperature-Growth Study

Temperature and mortality of juvenile blue crabs kept at a series of constant temperatures for 45 days are strongly correlated in this experiment (Figure 8). The correlation coefficient between temperature and percent mortality per sample is 0.923 for 14 samples ( $P < .01$ ) (Snedecor and Cochran, 1967). Mortality ranged from zero at 27 C to 80% at 35 C.

Within the range of temperatures in this experiment, the linear regression of mortality upon temperature was described by the equation  $Y = -254.014 + 9.3X$ , where Y is the percent mortality in 45 days and X is the acclimation temperature within the range 27-35 C.

Temperature had a significant effect upon the growth of juvenile blue crabs (Figures 9-10). Increasing temperature from 27 to 30 C provided increasing growth (Figure 9). Weight gain was optimal at 29-30 C. As temperature increased above 30 C (Figure 10), less growth was obtained. The samples at 31 C did not fit this pattern. They had much less growth than expected. Further evidence of the effect of temperature on growth was observed in the differences in the final total weights of samples (Table 3A) while no such differences occurred in the initial total sample weights (Table 3B).

The effect of temperature on growth of blue crabs was indicated by length of time taken by a sample of crabs to reach a certain weight

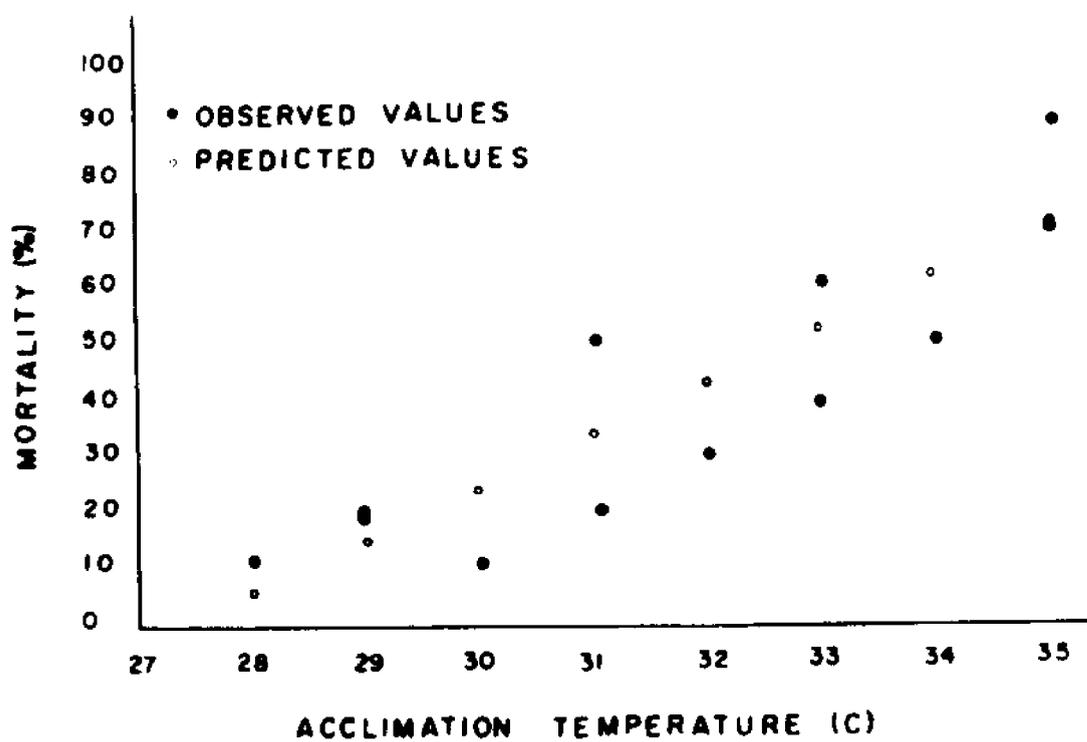


Figure 8. Correlation of mortality of juvenile blue crabs with temperature at the end of 45-day period. Linear regression equation:  $Y = -254.014 + 9.3X$ .

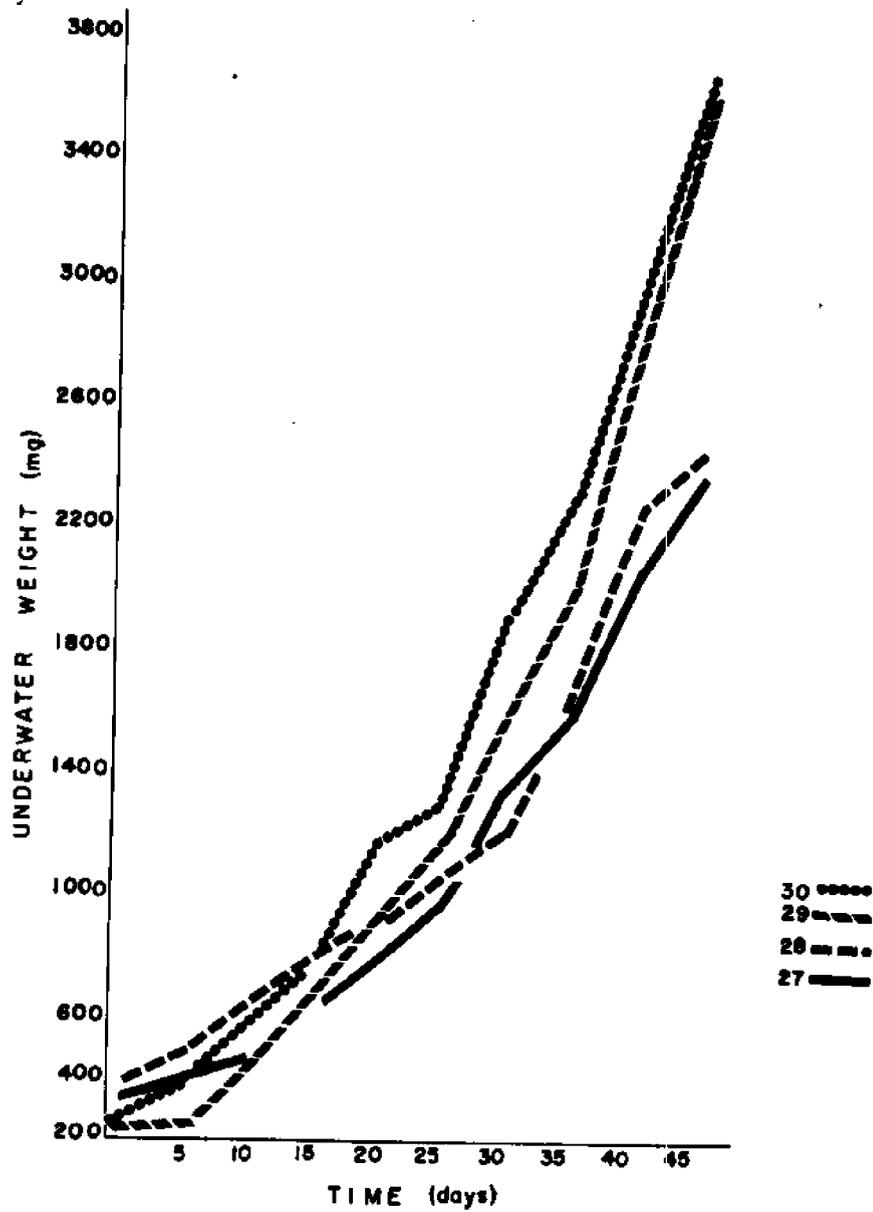


Figure 9. Comparative growth of juvenile blue crab groups at 30 C and below. All replicate data averaged.

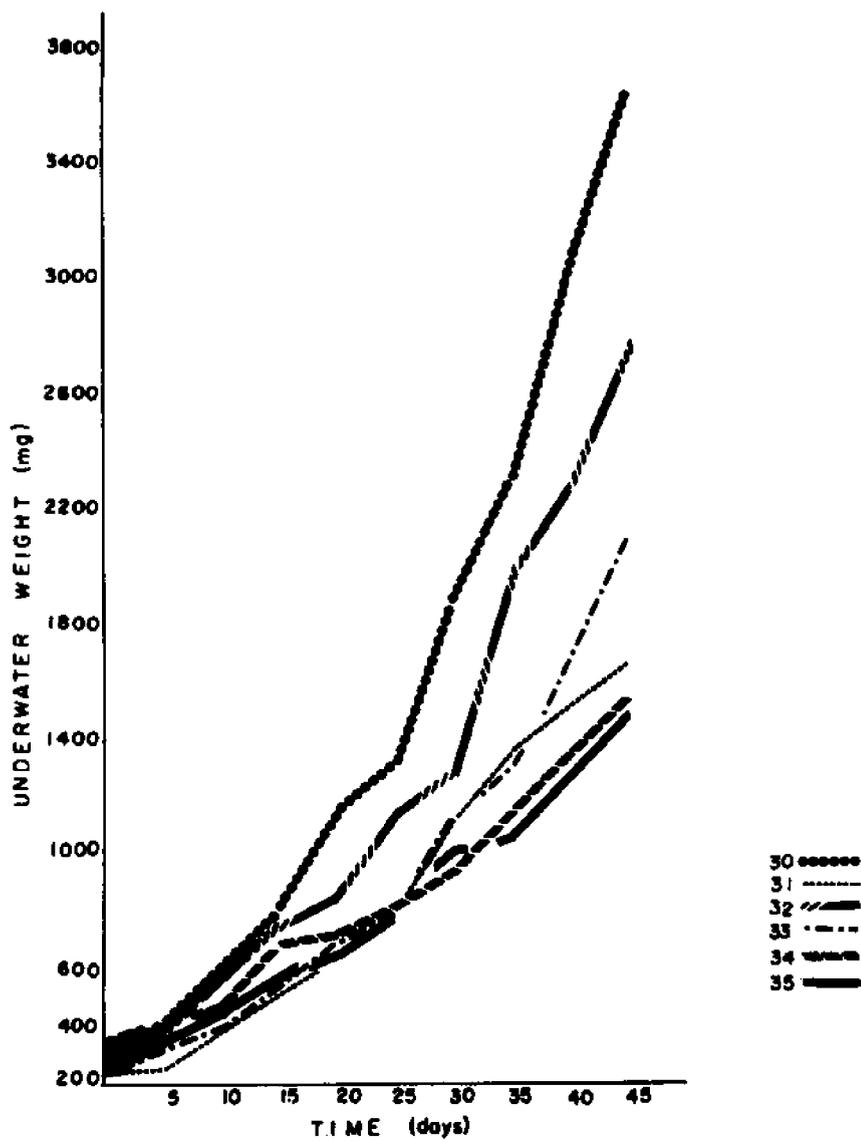


Figure 10. Comparative growth of juvenile blue crabs at 30 C and above. All replicate data averaged.

TABLE 3A.--Analysis of variance of the 45-day total weights of 14 groups of 10 crabs.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	139	2,823,109,393		
Treatment	13	1,014,568.693	78,043.746	5.437*
Error	126	1,808,540.700	14,353.498	

\*Significant ( $P < .05$ )

TABLE 3B. -- Analysis of variance of the initial total weights (ITW)  
of 14 groups of 10 crabs.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	139	359.39		
Treatment	13	49.47	3.80	1.55 <sup>1</sup>
Error	126	309.92	2.45	

<sup>1</sup> not significant

(Figure 11). The isopleths for time taken to reach the lower weight levels (600, 800 mg) are strongly influenced by the initial total weights (ITW) of the samples. As the experiment progressed, the ITW became less a factor. This may be observed in the 28, 29 and 30 C samples. The samples at 29 C reached 600 mg more slowly than those samples at 28 and 30 C. The ITW of the samples at 29 C was less than that of the samples at 28 and 30 C. As time progressed, however, the ensuing weights were reached by the sample at 29 C more quickly than the sample at 28 C and in about the same time as the 30 C sample. The isopleths representing time to reach the higher weights (1200, 1800 mg) would approximate normal curves except for the effect of the 31 C samples.

Comparison of the 14 final sample weights by Duncans New Multiple Range test (Li, 1964) indicates that the samples at 29 and 30 C grew significantly better than all other samples. One of the samples from 27 C and the one from 32 C were not significantly different from those at 29 and 30 C or from the other means. Li (1964) states that the status of overlapping means is uncertain. An analysis of variance, comparing the final total weights of both samples from 29 C with both samples from 27 C showed significant differences between growth at these temperatures (Table 4).

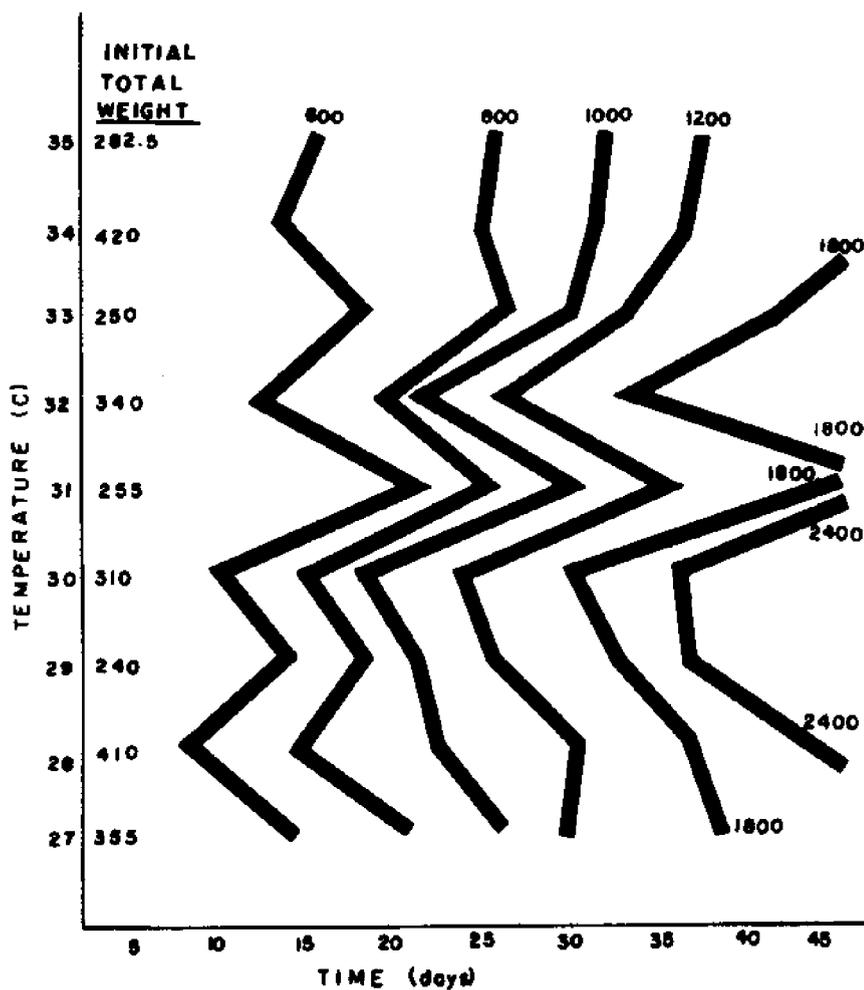


Figure 11. The effect of temperature upon growth of juvenile blue crabs as indicated by time required to reach a given weight. Numbers on isopleths indicate underwater weight (mg).

TABLE 4.--Analysis of variance of 45-day total weight of groups of crabs held at 27 and 29 C. Treatment: N = 20.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	39	762,709.1		
Treatment	1	134,792.1	134,792.1	8.1573*
Error	38	627,917.0		

\*Significant ( $P < .05$ )

Yield, the weight of the survivors from the original sample minus the weight of the original sample, was influenced by temperature in a 45-day period (Figure 12). The yield-temperature curve was generally similar to the final total weight-temperature curve. Mortality influenced yield (Figure 12) so that the temperatures at which yield deviates most noticeably from the final total weight were temperatures at which relatively high mortality occurred (33, 34, 35 C).

Yield was maximized at 29-30 C despite the fact that mortality at these temperatures was greater than that at lower test temperatures (Table 5).

All sizes of crabs were observed eating the pelleted feed mixture. The pellets lasted approximately 20 minutes in water. Small crabs did not consume the whole pellet which weighed approximately 66% as much as they did. Later in the experiment, the larger crabs did devour the pellet before it softened and dispersed. The crabs soon took the food eagerly and ate it somewhat untidily. The softened and dispersed food did not remain in the cages but settled to the bottom of the growth aquaria.

Food conversion efficiency was optimal at 29-30 C in this study (Table 6, Figure 13). Assuming that all food was consumed, maximum total food conversion efficiency for the 45-day study was 0.251. This is approximately a 4:1 food conversion ratio. Maximum food

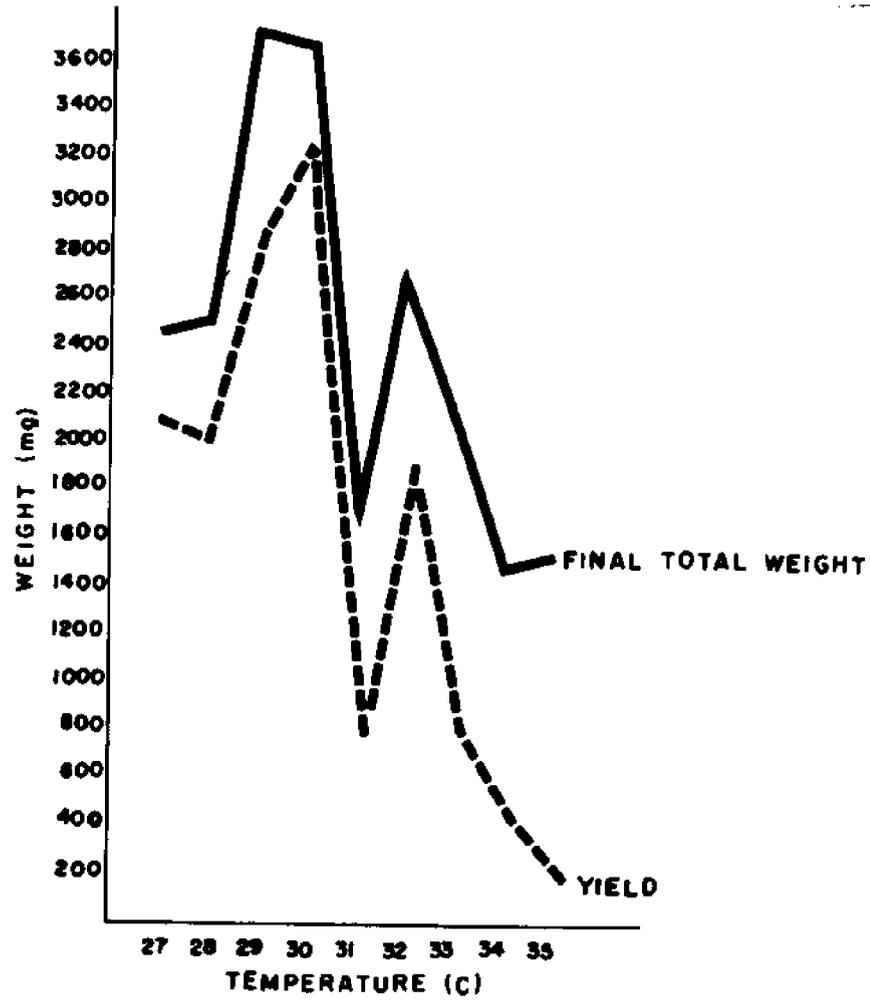


Figure 12. The effect of temperature on the final total weight and yield of groups of juvenile blue crabs.

TABLE 5. -- Summation of temperature-growth study data.

Tank	Temperature (C)	Initial total weight (mg)	Mortality (%)	Final total weight (mg) (corrected)	Yield (mg)	Food conversion efficiency
I	27	410	0	2,761	2,351	0.168
J	27	300	0	2,045	1,745	0.125
B	28	410	10	2,478	1,972	0.148
K	29	260	20	3,592	2,569	0.2315
L	29	220	20	3,730	3,047	0.251
D	30	310	10	3,674	3,194	0.241
M	31	270	50	2,128	713	0.133
N	31	240	20	1,225	685	0.071
F	32	310	30	2,772	1,776	0.176
O	33	255	40	1,859	916	0.115
P	33	245	60	2,347	489	0.168
H	34	420	50	1,445	355	0.077
Q	35	320	70	1,532	255	0.087
R	35	245	90	1,447	-35	0.086

TABLE 6. --Five-day period food conversion efficiency of juvenile blue crab groups at various temperatures.

Temperature (C)	Period								
	1	2	3	4	5	6	7	8	9
27	0.076	0.031	0.119	0.111	0.138	0.345	0.124	0.364	0.179
27	0.028	0.058	0.103	0.071	0.095	0.124	0.179	0.211	0.225
28	0.068	0.11	0.095	0.073	0.106	0.073	0.256	0.403	0.121
29	0.021	0.104	0.173	0.141	0.166	0.223	0.301	0.556	0.394
29	0.012	0.139	0.116	0.196	0.169	0.252	0.217	0.544	0.549
30	0.073	0.140	0.111	0.255	0.083	0.352	0.259	0.467	0.380
31	0.011	0.110	0.108	0.144	0.115	0.255	0.155	0.184	0.101
31	-0.011	0.062	0.081	0.016	0.029	0.198	0.146	-0.0097	0.110
32	0.076	0.090	0.119	0.058	0.195	0.150	0.370	0.206	0.282
33	0.046	0.039	0.114	0.062	0.034	0.243	0.029	0.172	0.262
33	0.080	0.011	0.104	0.123	0.055	0.241	0.161	0.254	0.270
34	0.055	0.024	0.156	0.012	0.053	0.077	0.128	0.124	0.053
35	0.130	0.023	0.114	0.062	0.034	0.243	-0.017	0.091	0.225
35	0.092	0.047	0.105	0.063	0.047	0.244	-0.018	0.104	0.097

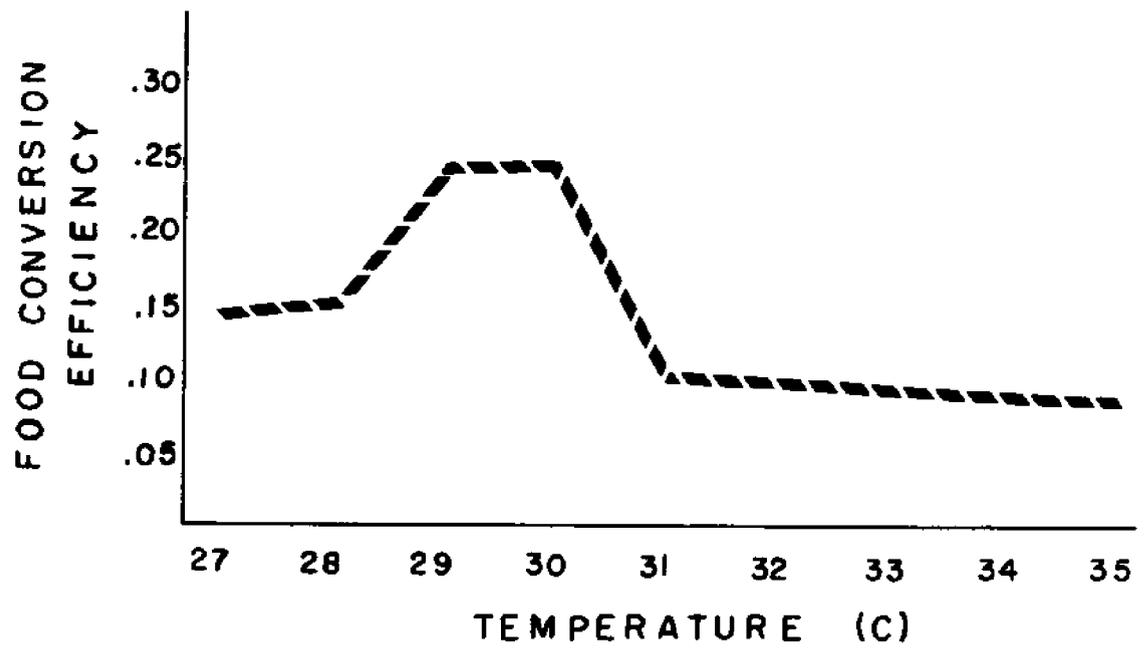


Figure 13. Food conversion efficiency of groups of juvenile blue crabs at various temperatures.

conversion efficiency for any 5-day growth period was 0.556, or a food conversion ratio of 1.78:1. Food conversion ratios of 3:1 were common in the later growth periods. The cumulative total food conversion efficiencies were neither as variable as the period food conversion efficiencies nor as high.

Molts were recorded daily. It was found that juvenile blue crabs may eat the cast-off exoskeleton within 6 to 8 hours after ecdysis. Therefore, many of the molts were probably not recorded. Several crabs were actually observed while molting. One took less than 3 minutes to withdraw from the old exoskeleton. When first observed, it was completely within the cracked exoskeleton. The juvenile blue crabs generally ate the cast-off exoskeleton completely, but often left the extreme tips of the chelepeda.

No effect of temperature on the number of molts was observed (Figure 14). Intermolt periods were calculated for all crabs which molted at least twice. The duration of the intermolt period apparently was affected by temperature (Figure 15). It appears that the duration of the intermolt period may be around 6-7 days at the optimum growth temperatures. Mean duration of the intermolt period at other temperatures appear to be from 8-12 days.

The initial total weights, within the range used in this study, show little effect on any of the parameters measured. There was no

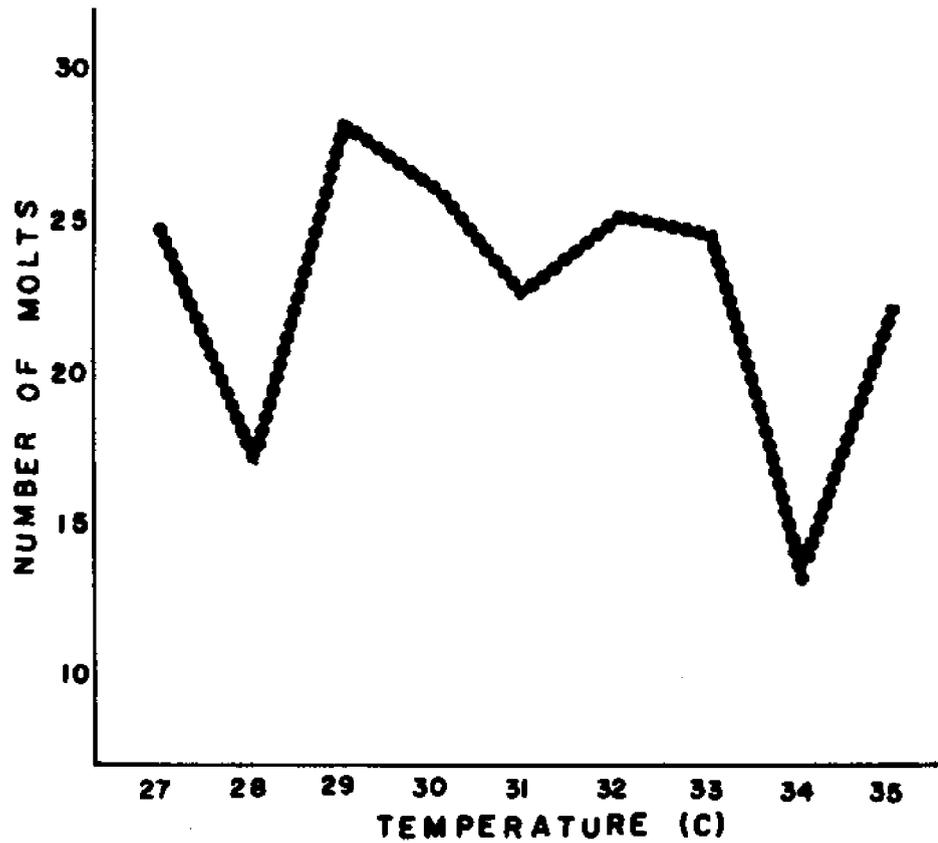


Figure 14. The average number of molts observed in groups of 10 small blue crabs at various temperatures during a 45-day period.

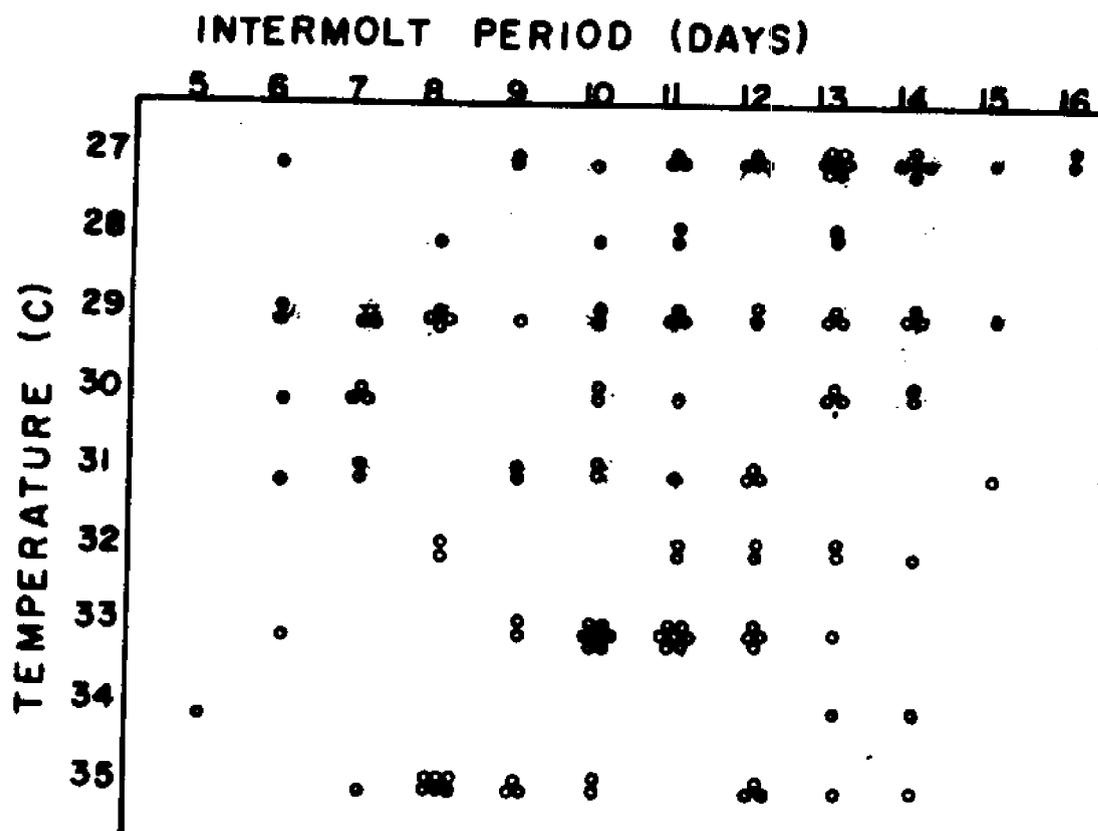


Figure 15. Duration of intermolt period of juvenile blue crabs maintained at various temperatures at 15‰ salinity.

significant correlation between initial total weights of the samples and final total weights, percent mortality, percent weight gain, or food conversion efficiency.

#### Salinity-Growth Study

Salinity had a significant effect on the mortality of small juvenile blue crabs (Figure 16). One hundred percent mortality was observed at 1‰ in the first 19 days of the study. Mortality was 10‰ or less during the same time period at all other salinities tested.

Water in the acclimation tanks containing the remaining eight groups was changed between the 19th and 22nd day. Mortality at all salinities began to increase (Figure 17). The increase in mortality appeared to be delayed for several days in the 6‰ samples.

On the 39th day, the water reservoir outside the laboratory was found to contain the remains of hundreds of dead polychaete worms and other marine invertebrates. This indicated water quality to be the reason for the crab mortality. The final mortality after 45 days was very high (70-90%) in all samples. The mean total mortalities at each salinity were not significantly different.

Salinity had no significant effect on the growth of juvenile blue crabs in this study (Figure 18). This does not include growth of crabs at 1‰ as those tests were stopped due to mortality. Analysis

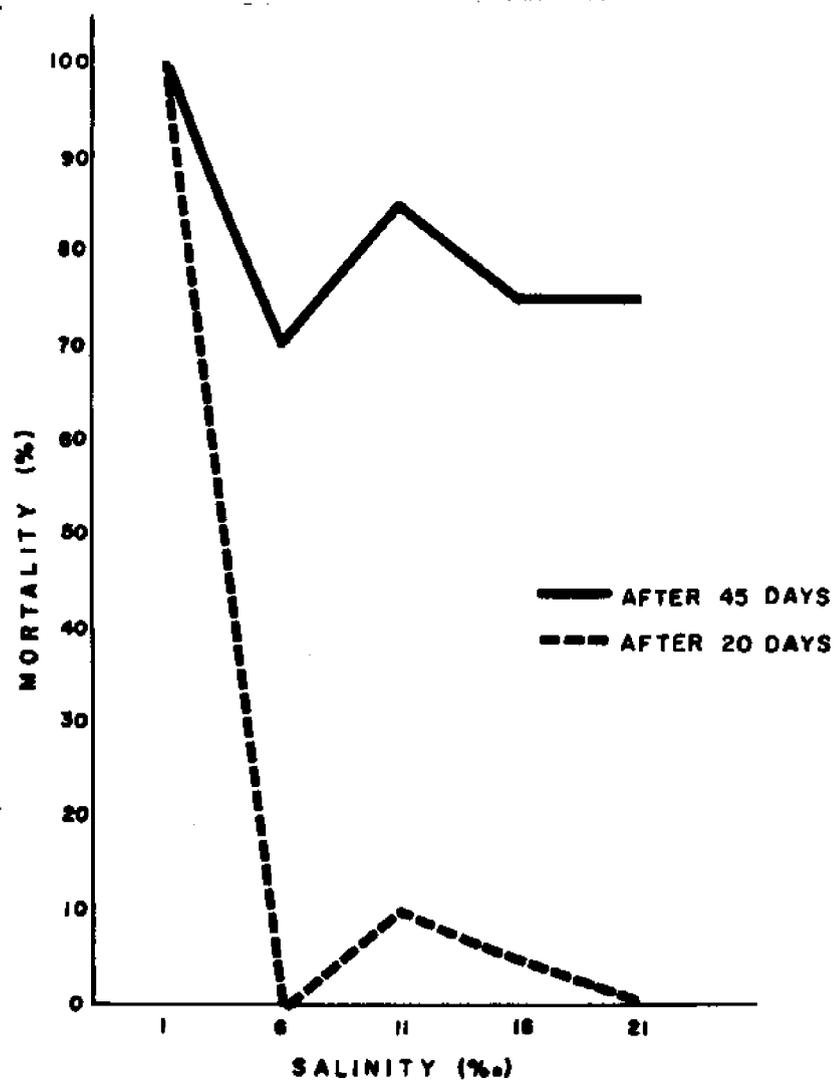


Figure 16. Mortality of juvenile blue crabs at various salinities before and after addition of suspect seawater.

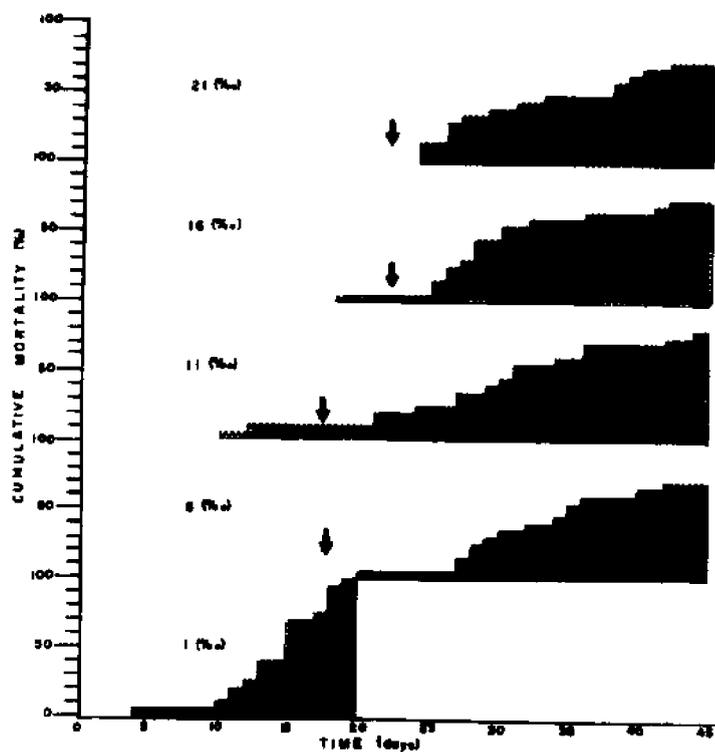


Figure 17. Cumulative mortalities of initially-stocked juvenile blue crabs in salinity-growth experiment. Arrows indicate date of water change in tanks of 6, 11, 16 and 21‰ salinity.

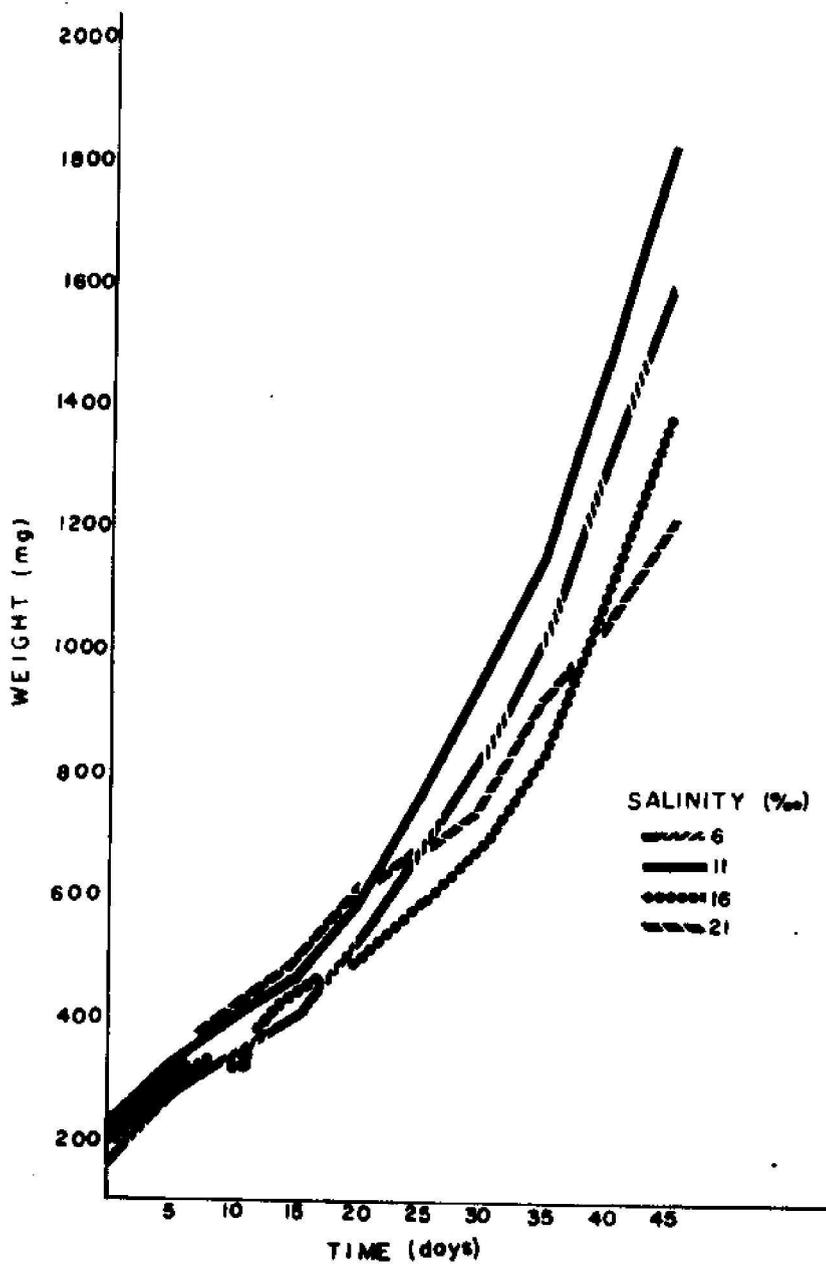


Figure 18. Growth of juvenile blue crab groups at 29 C in various salinities.

of variance of the average increment of gain per sample at each of the nine periods showed no significant differences between the samples from different salinities (Table 7A). Similar analysis was conducted on the data from the first four periods, prior to the mortality problem, and no significant differences between samples were detected (Table 7B).

There were no significant differences in sample weights after 20 days or at the termination of the experiment. Analysis of variance showed no differences between the weights of groups of crabs after 20 days (Table 8A) or after 45 days (Table 8B).

The extremely high rate of mortality made the analysis of yield in weight of survivors impractical.

Salinity had no effect on either the food conversion efficiency at each period or the cumulative food conversion efficiency (Figure 19). Analysis of variance of the food conversion efficiency at each period for the entire experiment (Table 9A) or for the initial 20 days (Table 9B) showed no significant differences between salinities. Food conversion efficiency is highly correlated with final total weight again in this study.

The most obvious effect of salinity upon molting of juvenile blue crabs is indicated by the high rate of mortality at 1‰ salinity. Each

TABLE 7A. --Analysis of variance of the weight increments of groups of small blue crabs held in different salinities (6, 11, 16 and 21‰). Weight increment was measured at 5-day intervals for 45 days.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	35	238,364.7		
Treatment (Salinity)	3	24,068.0	8,022.7	1.2 <sup>1</sup>
Error	32	214,296.7	6,696.8	

<sup>1</sup>not significant

TABLE 7B.--Analysis of variance of the weight increments of groups of small blue crabs held in different salinities (6, 11, 16 and 21‰). Weight increment was measured at 5-day intervals for 25 days.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	15	7,300.1		
Treatment (Salinity)	3	166.9	55.6	0.09354 <sup>1</sup>
Error	12	7,133.2	594.4	

<sup>1</sup> not significant

TABLE 8A.--Analysis of variance of the 45-day weights of groups of small blue crabs held in different salinities (6, 11, 16 and 21‰). Groups of 10 crabs were replicated in pairs at each salinity.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	79	391,287.9		
Treatment (Salinity)	7	62,914.8	8,987.8	1.97 <sup>1</sup>
Error	72	328,373.1	4,560.7	

<sup>1</sup>not significant

TABLE 8B. -- Analysis of variance of the 20-day weights of groups of small blue crabs held in different salinities (6, 11, 16 and 21‰). Groups of 10 crabs were replicated in pairs at each salinity.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	79	42,864.75		
Treatment (Salinity)	7	4,427.75	632.53	1.1849 <sup>1</sup>
Error	72	38,437.00	533.85	

<sup>1</sup>not significant

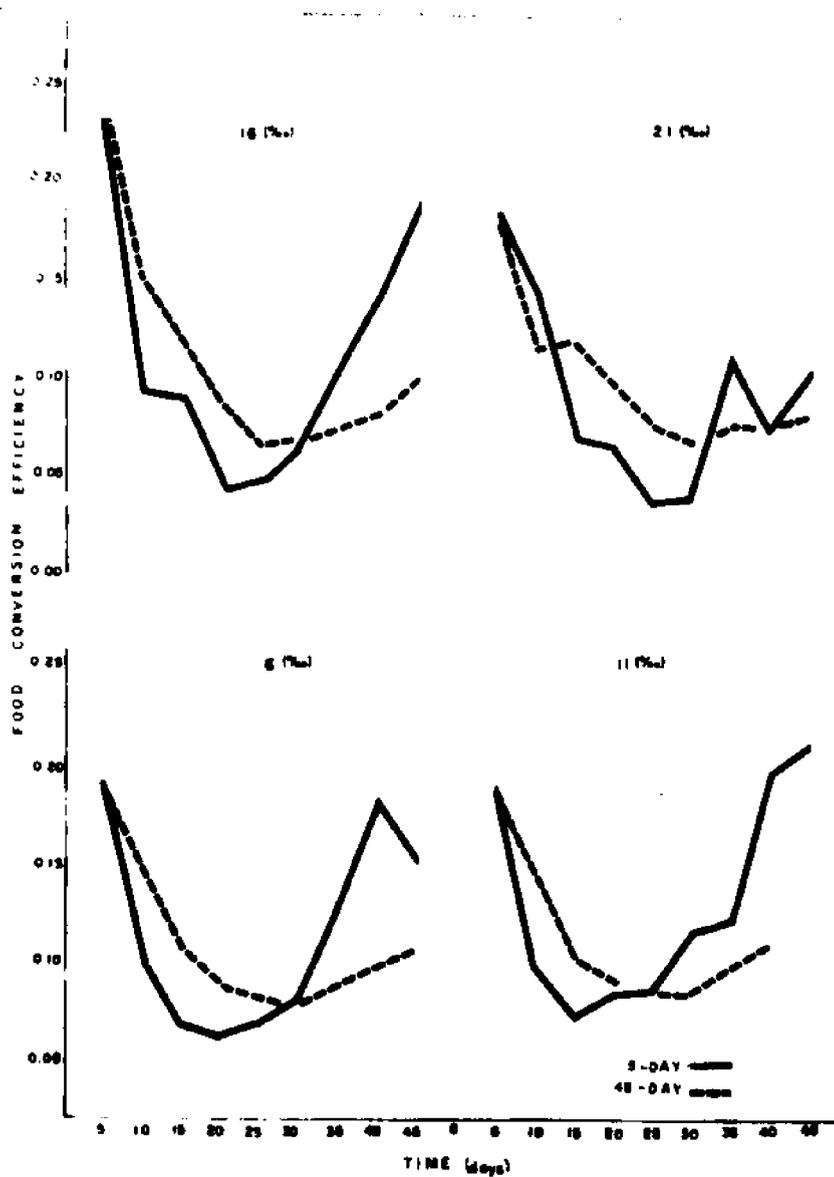


Figure 19. Cumulative and 5-day period food conversion efficiencies for groups of juvenile blue crabs in four salinities.

TABLE 9A.--Analysis of variance of nine consecutive 5-day food conversion efficiencies of groups of juvenile blue crabs held in different salinities (6, 11, 16 and 21‰).

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	25	91,835.18		
Treatment (Salinity)	3	7,538.16	2,512.71	0.95 <sup>1</sup>
Error	32	84,297.02	2,634.25	

<sup>1</sup> not significant

TABLE 9B.--Analysis of variance of four consecutive 5-day food conversion efficiencies of groups of juvenile blue crabs held in different salinities (6, 11, 16 and 21‰).

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	15	35,947.13		
Treatment (Salinity)	3	1,429.19	476.4	0.17 <sup>1</sup>
Error	12	34,517.94	2,876.5	

<sup>1</sup> not significant

of the crabs from these samples died during or shortly after ecdysis. The dead crabs appeared swollen or bloated.

The number of observed molts was not significantly different between groups of crabs at different salinities. Crabs were fed and observed twice daily during this study so that fewer molts should have been unrecorded.

There were no observable differences between the intermolt durations of groups of crabs at the four test salinities (Figure 20). Several short intermolt durations (3 and 4 days) were recorded.

#### Temperature-Salinity-Growth Study

Mortality of small crabs was approximately equal at both temperatures in the study (Figure 21). When the study was terminated, mortality at 15 C had just begun and had exceeded that at 29 C. At 29 C 40% of the crabs in 1‰ salinity died during the 30-day experiment. There was no mortality in other test salinities at 29 C during this period. However, factorial analysis of the mortality data from all salinity levels indicated no significant differences in mortality due to temperature alone (Table 10).

Small juvenile crabs died in about equal numbers at all test salinities (1, 2, 4 and 6‰) at the low temperature. Combining the mortalities within the salinity groups from both temperatures in

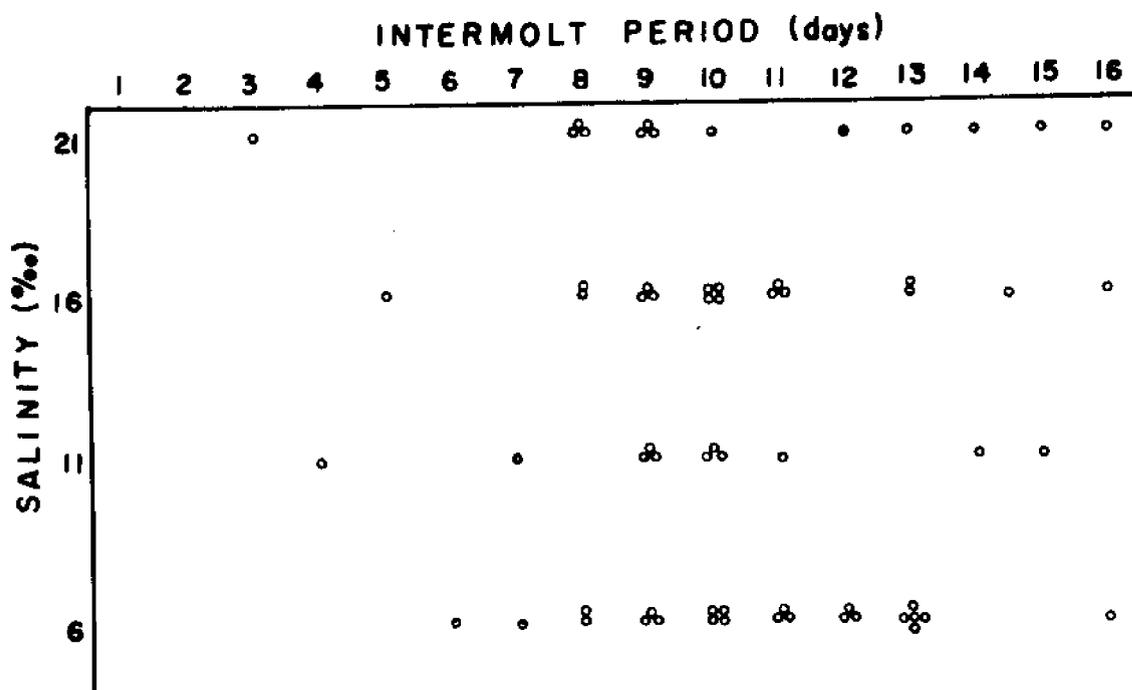


Figure 20. Duration of intermolt period of juvenile blue crabs maintained in four salinities (6-21‰) at 29 C.

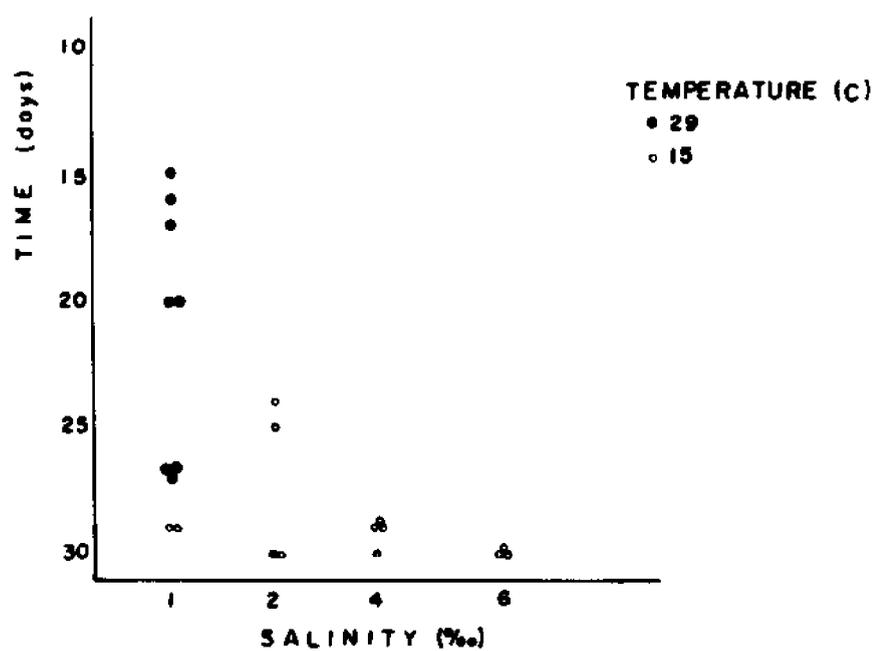


Figure 21. Daily mortality of juvenile blue crabs at 15 and 29 C and four salinities (1, 2, 4 and 6‰).

TABLE 10.--Analysis of variance from 2 x 4 x 6 factorial analysis of mortality data from groups of crabs maintained at different combinations of two temperatures (15, 29 C) and four salinities (1, 2, 4 and 6‰) for six consecutive 5-day intervals.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Replication	1	0.3750	0.375	3.1328 <sup>1</sup>
Period	5	8.9583	1.7817	14.9683**
Salinity	3	0.9166	0.3055	2.5522 <sup>1</sup>
Temperature	1	0.3750	0.3750	3.1328 <sup>1</sup>
Period-Salinity	15	2.9584	0.1972	1.6475 <sup>1</sup>
Temperature-Period	5	4.500	0.9000	7.5188**
Temperature-Salinity	3	2.3744	0.7915	6.6124**
Temperature-Salinity-Period	15	9.7503	0.6500	5.4302*
Error	47	5.6250	0.1197	

<sup>1</sup>not significant

\*(P < .05)

\*\* (P < .01)

factorial analysis indicated that salinity as a single, non-interacting factor, is not significant in mortality of small blue crabs within the test range (Table 10).

Time was a significant factor in the mortality of small blue crabs (Table 10). In general, more crabs died in the later period than in the early part of the study.

Significant first-order interactions, the interdependence of two factors in affecting a given parameter, exist between temperature, salinity and time in affecting mortality of juvenile blue crabs (Table 10). The lethal effects of salinity varied according to temperature and temperature produced mortality at different rates through time.

At 29 C, juvenile blue crabs were apparently killed by 1‰ salinity (Figure 22). Similar results were observed in the salinity-growth study. Comparisons were made on crabs maintained at 29 C at salinities of 1 and 6‰ in both of these studies. Analysis of variance indicated a difference between the salinities in producing mortality (Table 11). Mortalities occurred only at 1‰ at 29 C in both studies. At 15 C, 1‰ salinity produced no significant mortality. Analysis of variance of mortality among salinity samples at 15 C indicated no significant differences due to salinity (Table 12). No crabs died at 15 C for the first 24 days of the experiment, but 13 crabs died in the

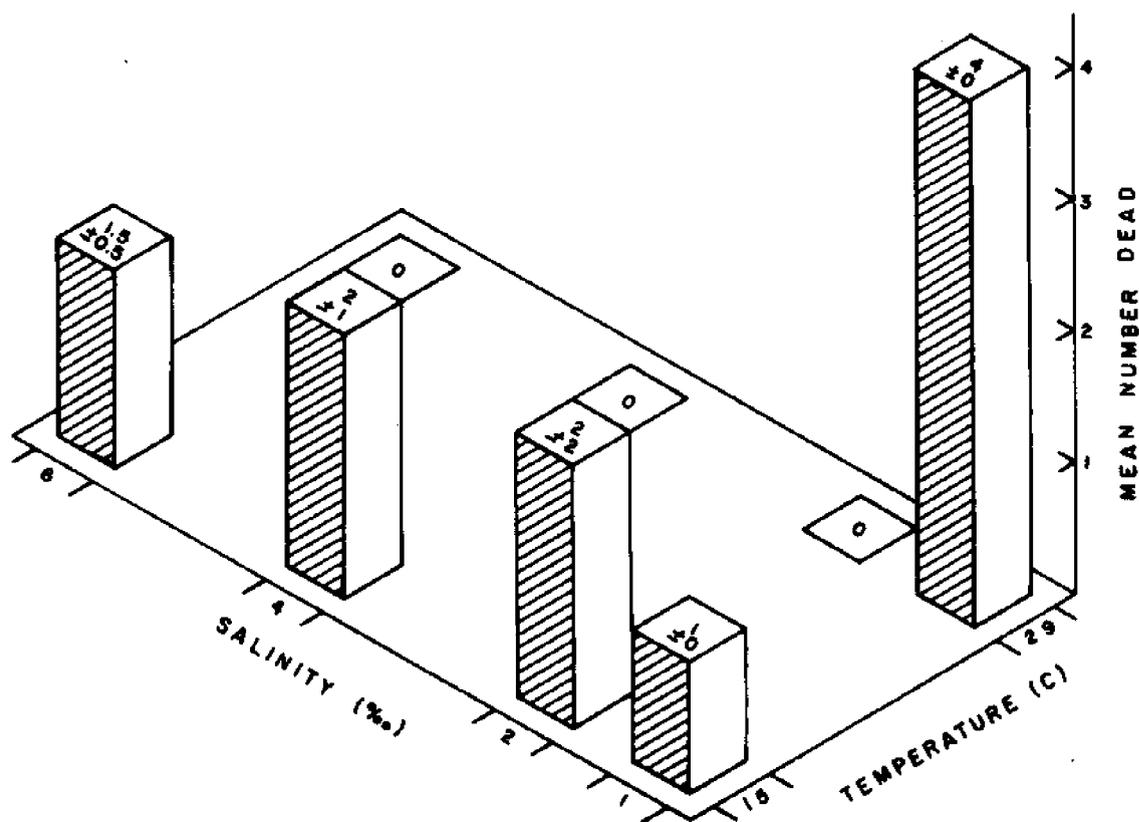


Figure 22. Thirty-day mortality of juvenile blue crabs at two temperatures and four salinities. For each test group,  $N = 10$ . Numbers on top of each histogram indicate mean and range of mortality of replicate groups at each combination of temperature and salinity.

TABLE 11.--Combination of mortality data from salinity-growth and temperature-salinity-growth studies. An analysis of variance of mortalities in 1 and 6‰ salinities at 29 C in both studies.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	7	134.9		
Treatment (Salinity)	1	78.5	78.5	8.4*
Error	6	56.4	9.4	

\* Significant ( $P < .05$ )

TABLE 12.--Analysis of variance of mortality in groups of juvenile blue crabs from four salinities (1, 2, 4 and 6‰) at 15 C.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	7	10		
Treatment (Salinity)	3	1	0.333	0.75001 <sup>1</sup>
Error	4	9	0.444	

<sup>1</sup>not significant

last 6 days. About 85% of those died in the last 2 days. No gross indications of disease or bad water quality were seen at this time. The significance of the temperature-time interaction (Table 10) is a result of this difference in the temporal distribution of mortality at 15 C.

Growth of juvenile blue crabs was affected by temperature, salinity and time within the ranges in this study (Table 13). Growth was most strongly affected by temperature (Figure 23). The mean final weights of groups of test animals maintained at 29 C exceeded those held in 15 C water. The important effect of temperature upon growth of small blue crabs was indicated at all periods during the study (Figure 24). Growth was better at 29 C throughout the study. Crabs maintained at 29 C grew better than those at 15 C in all salinities (Figures 23-24).

Growth of small crabs was also affected by salinity in this experiment (Table 13). Significant differences were indicated between crabs maintained in different salinities at 29 C (Table 14A) but not between those in different salinities at 15 C (Table 14B). At 29 C, crab growth was similar in all groups at 2, 4 and 6‰, but those groups held at 1‰ showed less growth (Figures 23-24). Comparison of the mean total weights of all groups of test animals at 29 C by a

TABLE 13. --Analysis of variance from 2 x 4 x 6 factorial analysis of weight increments of crabs maintained in combinations of two temperatures (15, 29 C) and four salinities (1, 2, 4 and 6‰) for six consecutive 5-day periods.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Replication	1	6,256.5104	6,256.5104	5.0048*
Period	5	38,787.7187	7,757.5437	6.2055**
Salinity	3	16,495.1979	5,498.3993	4.3983*
Temperature	1	296,259.2604	296,259.2604	236.9873**
Period-Salinity	15	26,825.2396	1,788.3493	1.4306 <sup>1</sup>
Temperature-Period	5	51,699.5521	10,339.9104	8.2712**
Temperature-Salinity	3	14,379.3646	4,793.1215	3.8342*
Temperature-Salinity-Period	15	34,212.5729	2,280.838	1.8245 <sup>1</sup>
Error	47	58,754.9896	1,250.1062	

<sup>1</sup>not significant

\*(P<.05)

\*\* (P<.01)

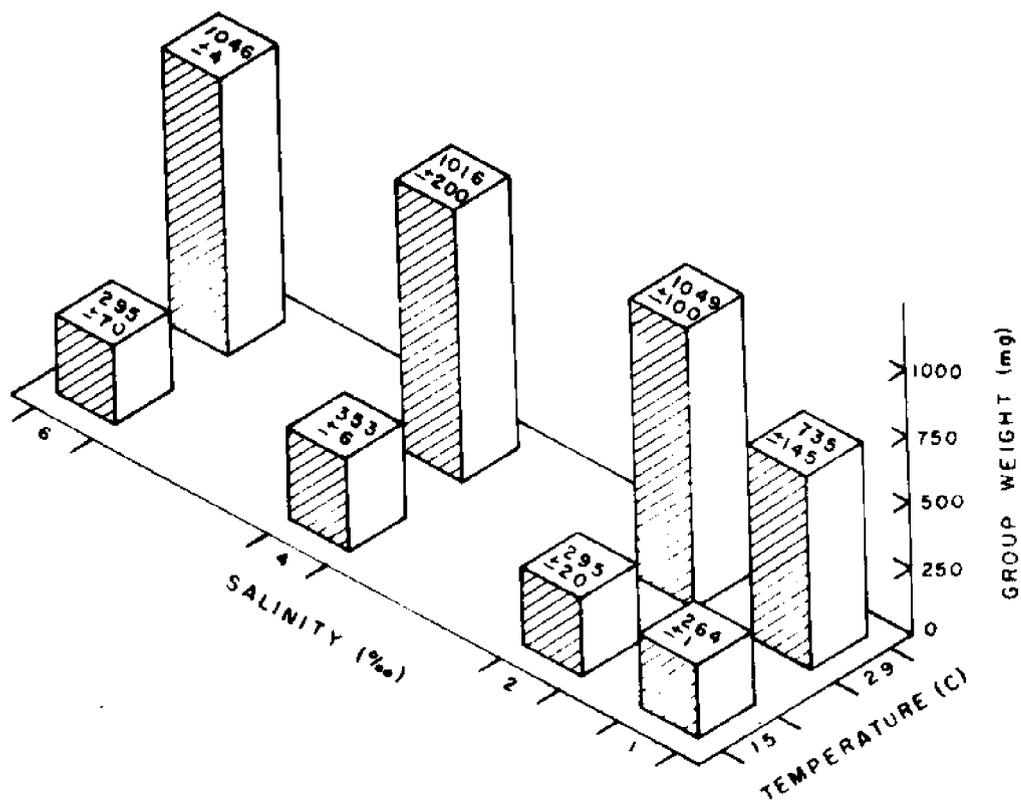


Figure 23. Mean 30-day growth of juvenile blue crabs at two temperatures and four salinities. For each test group,  $N = 10$ . Numbers on top of each histogram indicate growth mean and range for replicate groups at each combination of temperature and salinity.

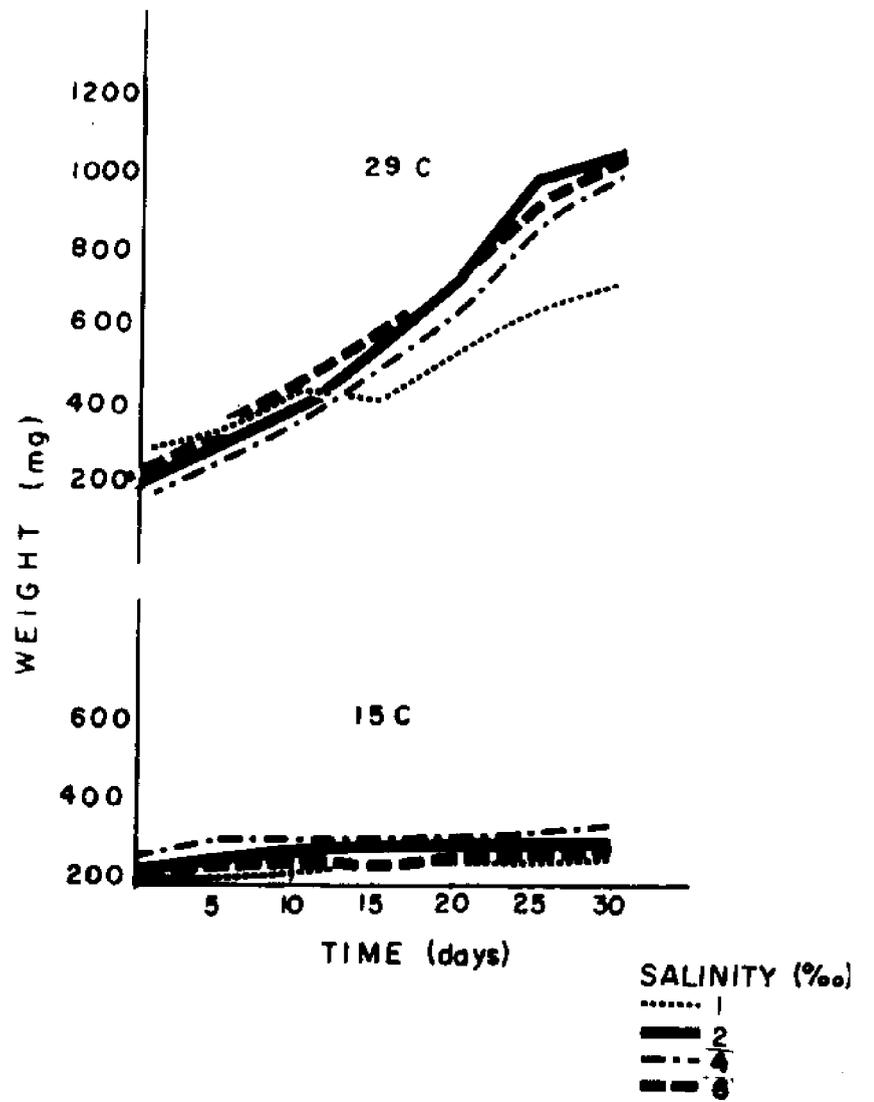


Figure 24. Weight increase of groups of juvenile blue crabs in four salinities at two temperatures.

TABLE 14A. --Analysis of variance of 30-day total weights of nine<sup>1</sup> groups of juvenile blue crabs held at 29 C.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	89	176,145.7889		
Between	8	73,830.4889	9,228.8111	7.3062**
Within	81	102,315.3000	1,263.1519	

<sup>1</sup>Only eight samples were used in factorial design; 1 of the 3 replicates of 1~~00~~ was not used at each temperature.

\*\*Significant ( $P < .01$ )

TABLE 14B.--Analysis of variance of 30-day weights of nine<sup>1</sup> groups of juvenile blue crabs held at 15 C.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	89	27,816.6222		
Between	8	2,117.4222	264.6788	0.8342 <sup>2</sup>
Within	81	25,699.2000	317.2741	

<sup>1</sup>Only eight samples were used in factorial design; 1 of the 3 replicates of *1<sub>10</sub>* was not used at each temperature.

<sup>2</sup>Not significant

Studentized Range Q test (Snedecor and Cochran, 1967) indicated that 1‰ salinity significantly hindered growth ( $P < .05$ ).

First order interactions were significant in affecting growth of small blue crabs (Table 13). The interaction between temperature and salinity has already been observed in the different effects of 1‰ on growth at different temperatures. Growth of juvenile blue crabs was affected by an interaction between time and temperature (Table 13).

The mean weight increments for each period at the two temperatures indicate the fluctuation in growth through time (Figure 25). At 29 C, growth rate was greatest during the fourth and fifth periods. A reduction of growth occurred during the sixth period at 29 C (Figures 24-25). Many similar reductions in the growth rate of individual crabs for one period were observed in earlier growth experiments. By plotting the mean weight increments of groups of crabs at each period for each temperature (Figure 26), the interaction between time and temperature is graphically demonstrated. The crossing of the period-weight lines between temperatures is indicative of interactions between temperature and time in affecting growth. Growth rates were generally higher in later periods at 29 C and in the first period at 15 C.

The daily weight change of individual small crabs was observed to vary from -0.091 mg to 3.46 mg. The mean weight increment of

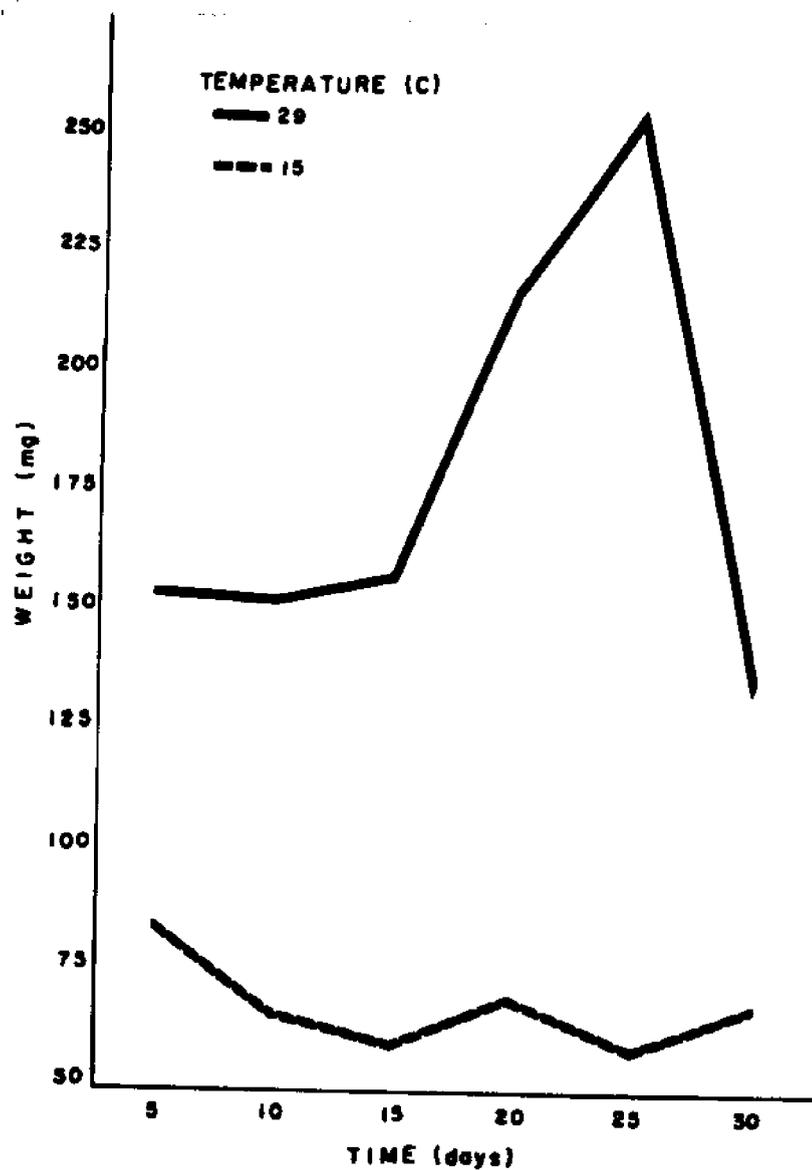


Figure 25. The effect of time on weight increments of juvenile blue crabs at 15 and 29 C.

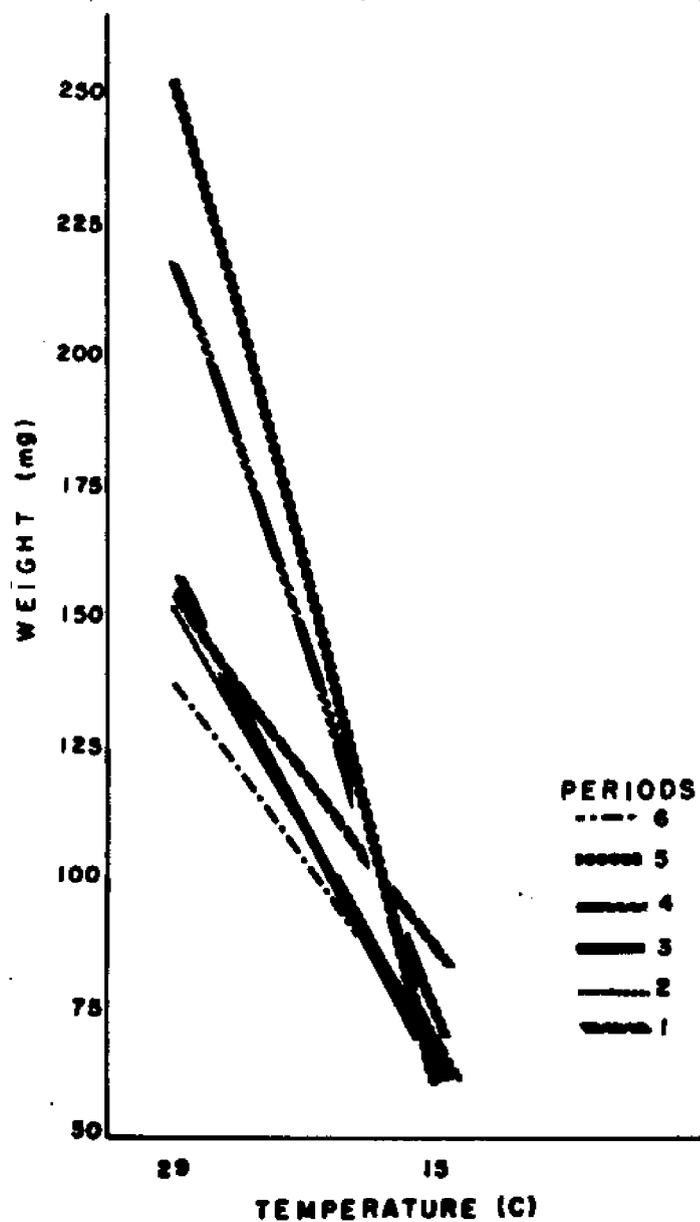


Figure 26. The effect of temperature on weight increments of juvenile blue crab groups during six consecutive 5-day periods.

crabs maintained at 29 C was 2.403 mg per day. This figure is based on the average increment of survivors from the original stock of each test group divided by the number of survivors and the number of days in the experiment. The corresponding figure for crabs maintained at 15 C was 0.152 mg per day. The average daily weight increment of individual crabs at 29 C was 15.8 times that observed for crabs maintained at 15 C.

Food conversion efficiency (FCE) for the 30-day experiment ranged from 0.105 to 0.0063. These figures are approximately equivalent to a 10:1 and a 150:1 food conversion ratio, respectively. Mean FCE at 29 C was 0.07606 (13:1) while mean FCE at 15 C was 0.00895 (111:1). Food conversion rates calculated for 5-day periods varied more widely. FCE for some 5-day periods reached 0.311 (3:1) at 29 C.

All major factors in this experiment (temperature, salinity and time) produced significant effects on food conversion efficiency of small blue crabs (Table 15). In general, FCE reacted similarly to growth. Since FCE is a function of growth and feeding rate and the feeding rate in this study was constant, the FCE should be closely correlated to growth.

Yield, the weight increase of survivors from the initial group above the ITW of the stock, was affected by growth and mortality

TABLE 15. ---Analysis of variance from a 2 x 4 x 6 factorial analysis of food conversion data of crabs kept in all combinations of four salinities (1, 2, 4 and 6‰) and two temperatures (15 and 29 C) for six consecutive 5-day periods.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Replication	1	3,434.138	3,434.138	8.4311**
Period	5	12,579.75	2,515.95	6.1769**
Salinity	3	6,868.2656	2,289.4219	5.6207**
Temperature	1	93,875.0417	93,875.0417	230.4721**
Period-Salinity	15	21,801.5782	1,453.4385	3.5683**
Temperature-Period	5	15,612.0333	3,122.4067	7.6658**
Temperature-Salinity	3	1,067.38	355.993	0.8740 <sup>1</sup>
Temperature-Salinity-Period	15	3,712.9721	247.5315	0.6077 <sup>1</sup>
Error	47	19,143.867	407.3163	

<sup>1</sup> not significant

\*\* (P < .01)

(Figure 22). Yield is directly related to growth and is inversely related to mortality in this study (Figures 22, 23 and 27).

Yield was significantly different between crabs at 29 C and 15 C (Table 16A and Figure 27). Combining the salinity data from both temperatures in analysis of variance detected no differences in crab yield due to salinity. Analysis of variance of yields in various salinities at 29 C is very close to significant at 95% level (Table 16B). Apparently crabs at 1‰ salinity have lower yields (Figure 27). Salinity groups at 15 C did not differ significantly in terms of yield (Table 16C).

#### Mortality-Substrate Study

Mortality in 18 test groups of 50 uncaged juvenile blue crabs was observed under different combinations of temperature and substrate for several periods of time. Cannibalism was observed at all combinations of temperature and substrate throughout the study. It appeared to be the major cause of mortality in these uncaged crabs.

The numbers of survivors after 15 and 25 days in each of the nine experimental groups were examined by factorial analysis (Table 17). Mortality was apparently affected by the three factors, temperature, substrate and time. Variance in survival within replicates of the same test conditions and between groups at different temperatures or

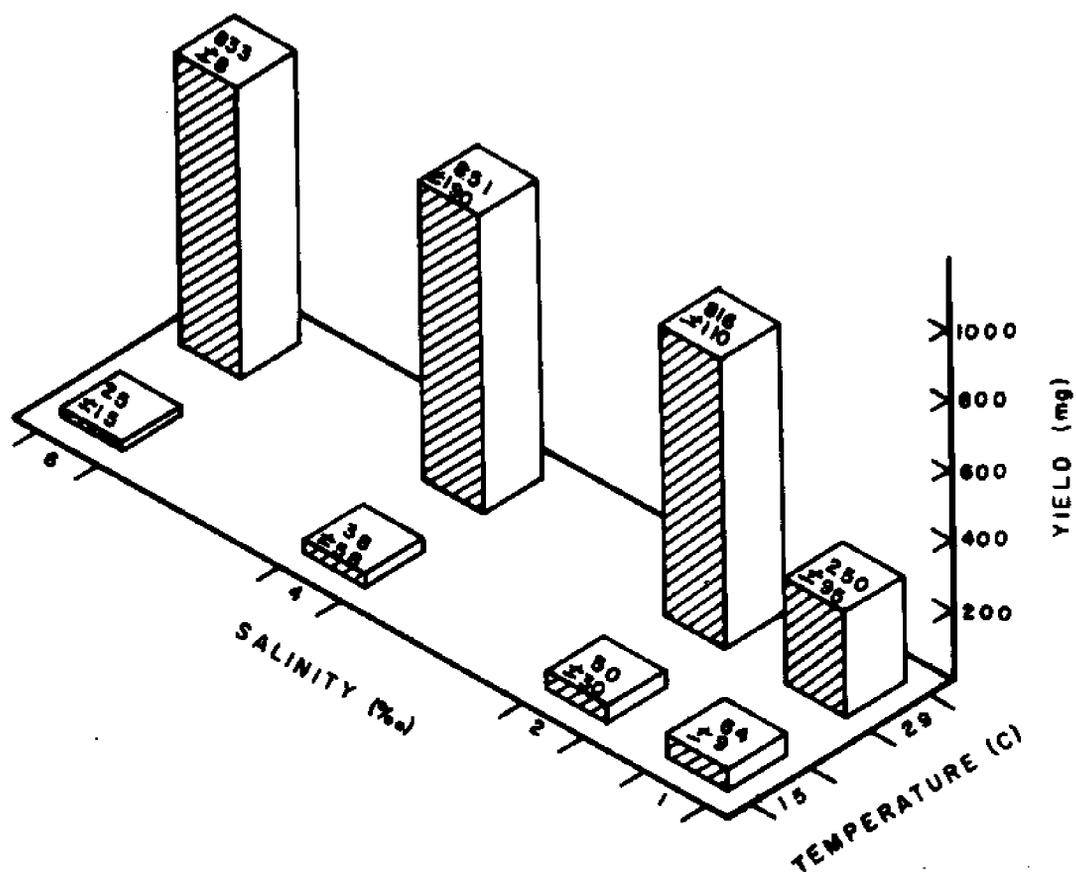


Figure 27. Mean 30-day yield of groups of juvenile blue crabs in four salinities at two temperatures. Numbers on top of each histogram indicate yield mean and range for replicate groups at each combination of temperature and salinity.

TABLE 16A.--Two-way analysis of variance of yield from groups of blue crabs. Data are from groups of crabs kept at all combinations of two temperatures (15, 29 C) and four salinities (1, 2, 4 and 6‰).

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	7	1,087,787.5		
Temperatures	1	832,050.0	832,050.0	17.78 <sup>1</sup>
Salinity	3	115,394.5	38,464.83	0.82
Error	3	140,343.0	46,781.0	

<sup>1</sup>Significant ( $P < .025$ )

TABLE 16B.--Analysis of variance of yield from groups of blue crabs maintained in four salinities (1, 2, 4 and 6‰) at 29 C.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	7	621,346		
Salinity	3	509,311	169,770.33	6.06133 <sup>1</sup>
Error	4	112,035	28,008.75	

<sup>1</sup>Significant ( $P < .06$ )

TABLE 16C.--Analysis of variance of yield from groups of blue crabs maintained in four salinities (1, 2, 4 and 6‰) at 15 C.

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	7	10,556.0		
Salinity	3	1,746.0	582	0.26425 <sup>1</sup>
Error	4	8,810.0	2,202.5	

<sup>1</sup>not significant

TABLE 17. --Analysis of variance from 2 x 3 x 3 factorial design analysis of the number of survivors after 15 and 25 days from groups of uncaged juvenile blue crabs held at different combinations of temperatures (20, 25 and 30 C) and substrate types (glass, sand, and sand-plus-shell).

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	35	6,819.222		
Replication	1	544.444	544.444	9.177*
Time	1	1,681.00	1,681.00	28.3345*
Temperature	2	2,477.72	1,238.86	20.8819*
Substrate	2	654.055	327.0275	5.5123*
Time-Temperature	2	60.1689	30.0845	0.5071 <sup>1</sup>
Time-Substrate	2	22.1665	11.0883	0.1868 <sup>1</sup>
Temperature-Substrate	4	327.4465	81.8616	1.3798 <sup>1</sup>
Time-Temperature-Substrate	4	13.6701	3.4175	0.0576 <sup>1</sup>
Error	17	1,008.558	59.3269	

<sup>1</sup>not significant

\*significant (P < .05)

substrates appeared large. Bartlett's test of homogeneity of variance indicated that no significant heterogeneity of variances (heteroscedasticity) of survival data from the different temperatures ( $P < .05$ ; Table 18). Similar analysis indicated no significant differences in variance of survival data between substrate groups ( $P < .05$ ; Table 19).

Graphic representation of the survival data from the various test groups tends to support the differences due to temperature and substrate (Figure 28). It appears that as temperature increased, survival decreased and that sand-plus-shell substrate was superior to either sand or glass in promoting survival of small crabs.

Approximately 58% of the crabs survived for 25 days at 20 C while 30% and 11% survived at 25 and 30 C, respectively (Figure 29). Comparisons of the mean survival from each temperature by Studentized Range Q test (Snedecor and Cochran, 1967) indicate that survival at 20 C differed significantly ( $P < .05$ ) from survival at 30 C.

Better survival of small crabs occurred in sand and shell than in the other substrates (Figure 30). When survival of all temperature groups was combined, there appeared to be no significant protection from a layer of sand compared to bare glass. Comparison of survival in each of these two substrates at each temperature indicated possible temperature-substrate interactions (Figure 28). At 20 and 30 C, glass

TABLE 18.--Bartlett's test for homogeneity of variance of mortality data from 18 groups of 50 uncaged juvenile blue crabs held at three temperatures (20, 25 and 30 C) for 25 days.

	Number survivors after 25 days		
	20 C	25 C	30 C
	33	17	9
	41	30	10
	21	6	1
	24	24	3
	17	2	2
	37	9	8
$\chi^2$	5445.0	1886.0	259.0
x	173	88	33
SS	456.833	595.333	77.50
Independent estimate of variance	91.366	119.066	15.5

pooled estimate of variance 75.31

$$B = (1.87685) (15) = 28.15275$$

$$M = 6.96$$

$$C = 1.3555$$

$$\chi^2_2 = 5.14 \text{ not significant}$$

TABLE 19.--Bartlett's test for homogeneity of variance of mortality data from 18 groups of 50 uncaged juvenile blue crabs held on three substrates (glass, sand, sand-plus-shell) for 25 days.

	Number of survivors after 25 days		
	Glass	Sand	Sand-plus-shell
	17	21	33
	37	24	41
	2	6	17
	9	24	30
	2	1	9
	8	3	10
$x^2$	4140.0	1639.0	1881.0
x	140	79	75
SS	873.3	598.8	873.5
Independent estimate of variance	174.70	119.77	174.67

pooled estimate of variance 156.3773

$$B = (2.19424) (15) = 32.91360$$

$$M = 0.20201$$

$$C = 1.3555$$

$$X_2^2 = 0.14903 \text{ not significant}$$

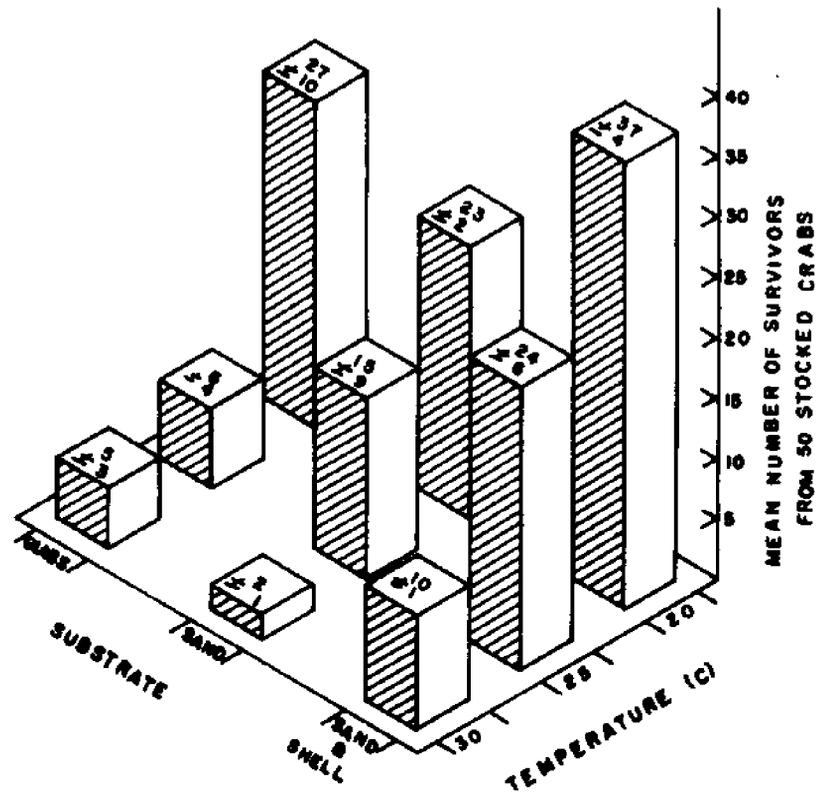


Figure 28. Mean 25-day survival of uncaged juvenile blue crabs at three temperatures and three substrates. Numbers on top of each histogram indicate the survival mean and range for replicate groups of 50 crabs at each temperature-substrate combination.

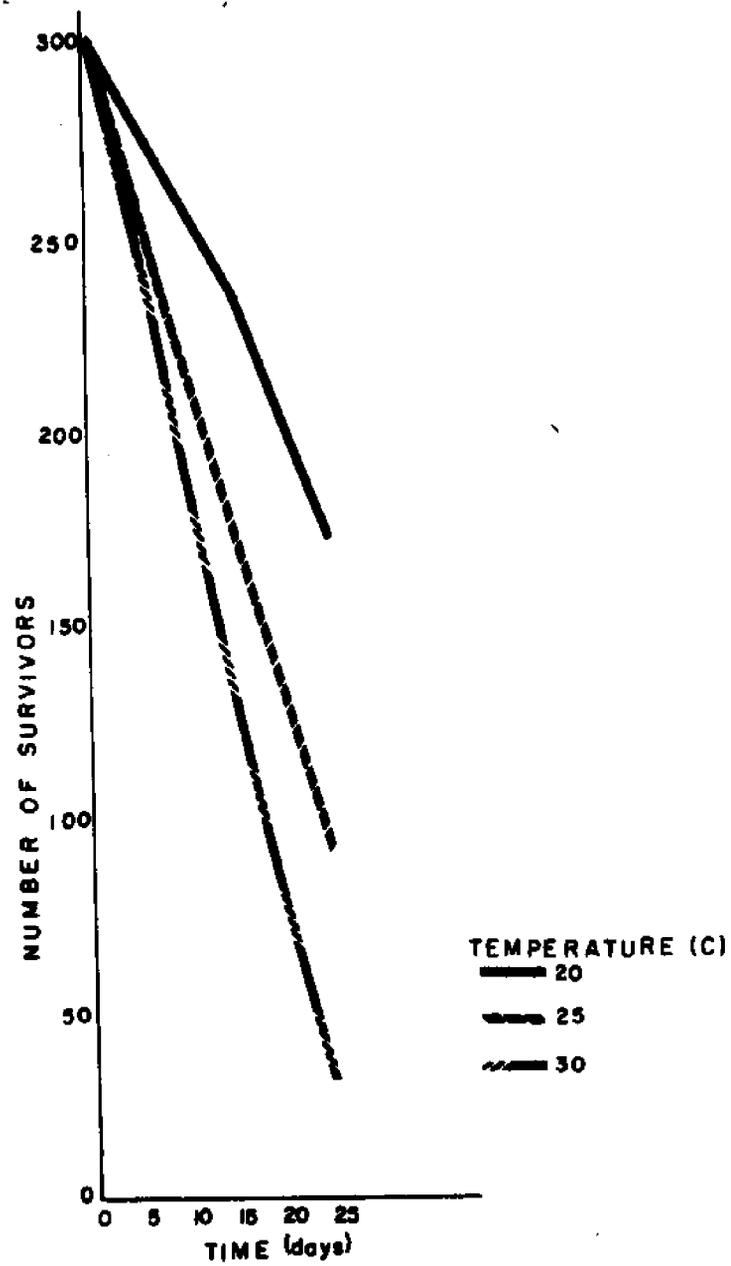


Figure 29. The effect of temperature on the survival of uncaged juvenile blue crabs.

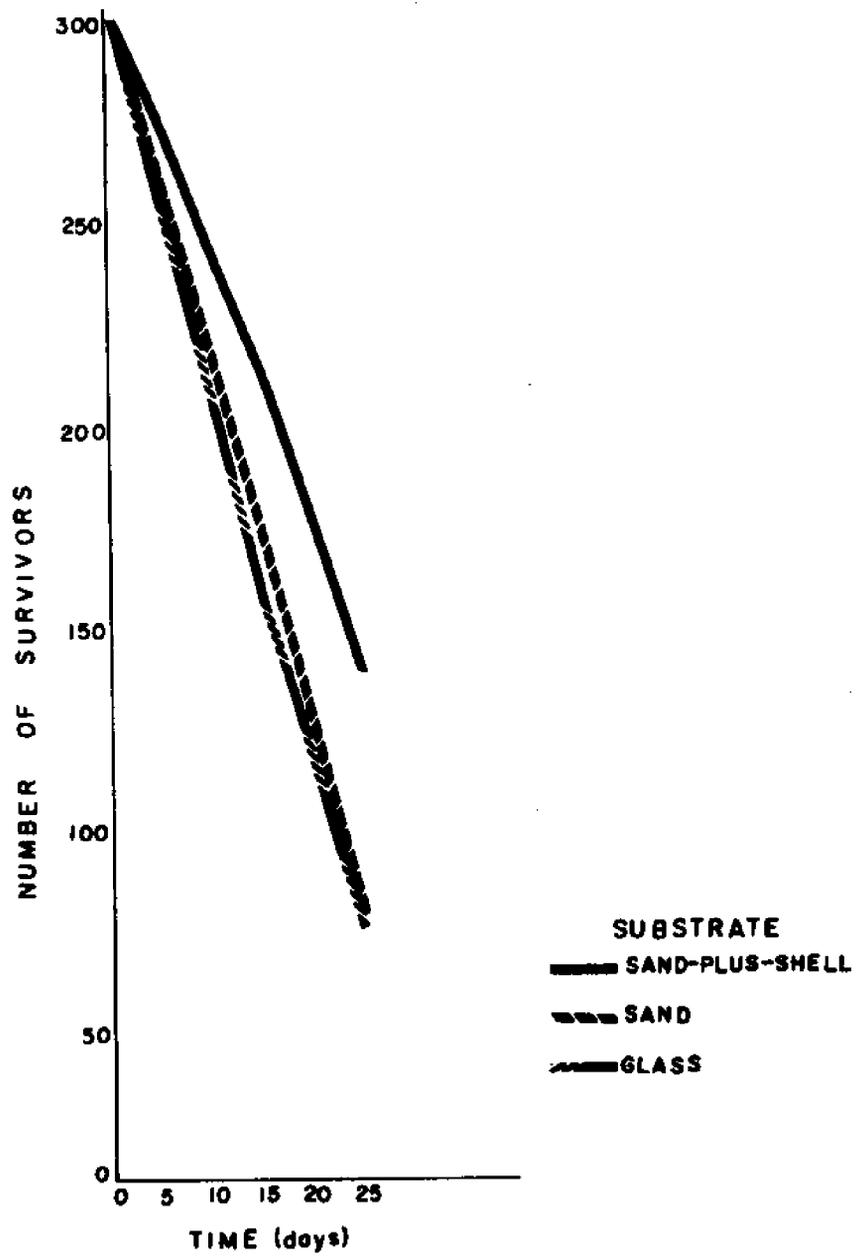


Figure 30. The effect of substrate upon the survival of uncaged juvenile blue crabs.

provided better survival than sand, while at 25 C sand only provides best survival. This interaction is not indicated between either glass or sand only and the sand-plus-shell substrate. Factorial analysis of the mortality data indicates no significant interactions between any of the major factors in this study (Table 17).

Differences in the variation within temperature groups particularly in one replicate of the sand substrate at 30 C and one of the control substrate at 20 C were noted in conjunction with this apparent interaction between control and sand only substrates with temperature.

Mortality increased through time in this study. Time was indicated as a significant factor in small blue crab mortality (Table 17). The mortality rate, as indicated by the slopes of survival lines in Figures 29 and 30, did not change through time. The unchanging slope of survival lines also indicated the lack of time-temperature interactions (Figure 29) and time-substrate interaction (Figure 30).

Yield of blue crabs in this study was directly related to mortality (Figure 31). Generally, yield increased with mortality except in some replicates in which heterogeneity of variance (heteroscedasticity) was suspected.

Bartlett's test for homogeneity of variance indicated heteroscedasticity for groups in different temperatures (Table 20) and different substrates (Table 21). Logarithm transformation of the yield

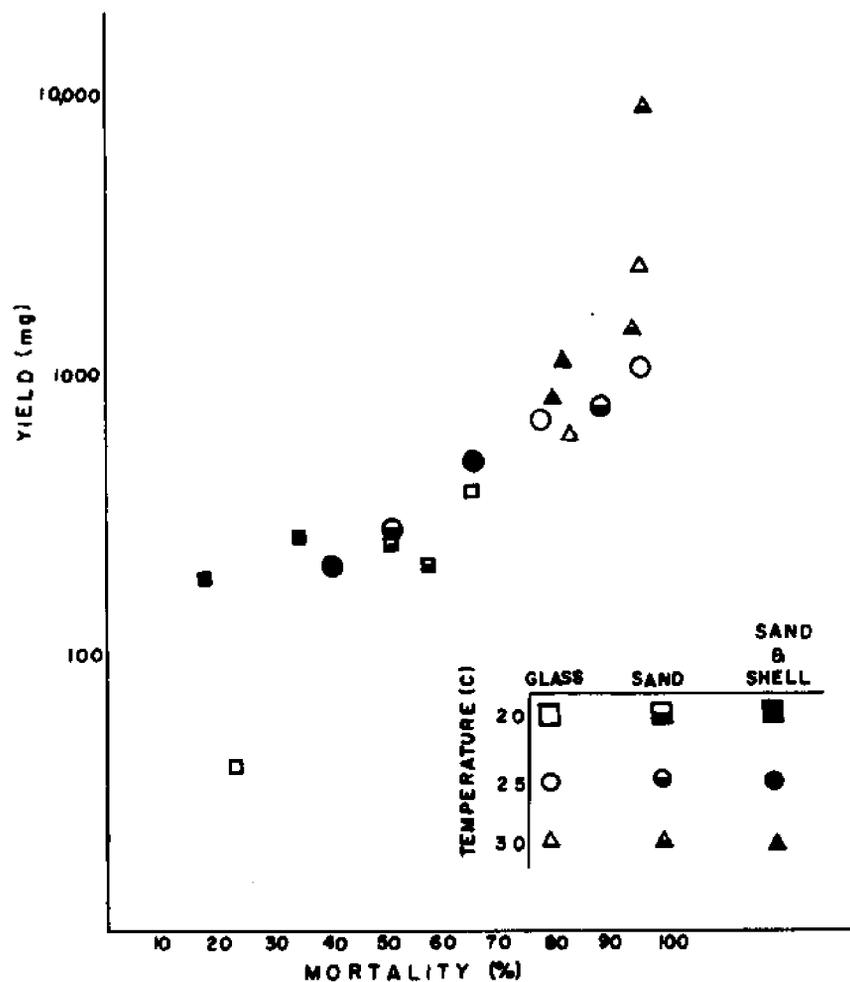


Figure 31. Relationship between mortality and yield of uncaged juvenile blue crabs in nine temperature-substrate combinations. Each symbol represents data from an initial group of 50 crabs. Replicate groups are indicated.

TABLE 20.--Bartlett's test for homogeneity of variance of yield data from 18 groups of 50 uncaged juvenile blue crabs held at three temperatures (20, 25 and 30 C) for 25 days.

	Yield in milligrams after 25 days		
	20 C	25 C	30 C
	347.8	1069.8	2547.8
	254.3	797.1	9411.6
	268.0	495.1	836.8
	42.2	668.0	624.6
	208.6	258.3	1428.5
	184.9	206.3	1121.1
$x^2$	336,940.14	2,580,467.04	99,456,336.26
$x$	1,305.8	3,494.6	15,970.40
SS	52,754.5	545,095.5	11,389,678.1
Independent estimate of variance	10,550.9	109,019.1	11,389,678.1

pooled estimate of variance 3,836,416.02

$$B = (6.58388) (15) = 98.75820$$

$$M = 41.79484$$

$$C = 1.3550$$

$$x^2_2 = M/C = 30.83352 \quad \text{significant (P < .001)}$$

TABLE 21.--Bartlett's test for homogeneity of variance of yield data from 18 groups of uncaged juvenile blue crabs held on three substrates (glass, sand, and sand-plus-shell) for 25 days.

	Yield in milligrams after 25 days		
	Glass	Sand	Sand-plus-shell
	374.8	254.3	268.0
	42.2	208.6	184.9
	1069.8	797.1	495.6
	668.0	258.3	206.3
	2547.8	9411.6	836.8
	624.6	1428.5	1121.1
$\chi^2$	8,614,361.9	91,429,096.6	2,351,290.5
x	5,327.2	12,358.4	3,112.7
SS	3,884,518.6	65,974,088.1	736,473.6
Independent estimates of variance	776,903.7	13,194,817.6	147,294.7

pooled estimate of variance 4,706,338.7

$$B = 100.09$$

$$M = 23.5$$

$$C = 1.3555$$

$$\chi^2_2 = 17.3616 \text{ significant (P < .001)}$$

from each test group provided homogeneity of variance within temperature groups (Table 22) and within substrate groups (Table 23). Statistical procedures were calculated on the logarithm-transformed data.

Yield of small blue crabs was significantly effected by temperature (Table 24, Figure 32). Crabs maintained at 30 C were more productive than those at 20 C ( $P < .05$ ) and 25 C ( $P < .05$ ). At 25 C, yield was greater than that at 20 C ( $P < .06$ ) (Table 25).

Substrate apparently had no significant effect on yield of small blue crabs (Table 23, Figure 31). However, more crabs survived in the sand-plus-shell substrate test groups than in other substrate test groups and the inverse relation between mortality and yield has been noted.

Food conversion efficiencies (FCE) in this study, the yield in wet-in-air weight divided by the amount of food fed, were very low. In the group that had only one survivor, food conversion efficiency was high (0.32034). This is approximately a 3:1 food conversion ratio. The one crab that survived in that sample weighed more than all other 30 C survivors combined. All other FCE were less than 0.1 or a 10:1 food conversion ratio. This was also true of yield and number of survivors since food conversion efficiency is dependent upon yield.

TABLE 22.--Bartlett's test for homogeneity of variance of the logarithm-transformation of yield data from 18 groups of 50 uncaged juvenile blue crabs maintained at three temperatures (20, 25 and 30 C) for 25 days.

	Logarithm of yield (milligrams)		
	20 C	25 C	30 C
	2.57	3.02	3.40
	2.40	2.90	3.97
	2.42	2.69	2.92
	1.62	2.82	2.79
	2.31	2.41	3.15
$x^2$	31.28	43.86	62.79
x	13.58	16.15	19.27
SS	0.55	0.39	0.90
Independent estimate of variance	0.11	0.07	0.18

pooled estimate of variance 0.12

$$B = -13.66$$

$$M = 0.89$$

$$C = 1.3555$$

$$X_2^2 = 0.65 \text{ not significant}$$

TABLE 23. -- Bartlett's test for homogeneity of variance of the logarithm-transformed yield data from 18 groups of 50 uncaged juvenile blue crabs maintained on three substrate types (glass, sand and sand-plus-shell) for 25 days.

	Logarithm of yield (milligrams)		
	Glass	Sand	Sand-plus-shell
	2.57	2.42	2.31
	2.40	1.62	2.26
	3.02	2.69	2.41
	2.90	2.82	2.31
	3.40	2.92	3.15
	3.97	2.79	3.04
$x^2$	57.21	39.9	40.7
$x$	18.26	15.2	15.4
SS	1.64	1.10	0.80
Independent estimate of variance	0.328	0.220	0.160

pooled estimate of variance 0.236

$$B = -9.406$$

$$M = 0.647$$

$$C = 1.3555$$

$$X_2^2 = 0.478 \text{ not significant}$$

TABLE 24.--Randomized blocks analysis of variance of logarithm-transformed yield data from 18 groups of 50 uncaged juvenile blue crabs at three temperatures (20, 25 and 30 C) and two replicates of three substrates (glass, sand, and sand-plus-shell).

Source of variation	Degree of freedom	Sums of squares	Mean square	F
Total	17	4.55		
Treatment (temperature)	2	2.70	1.35	17.13**
Blocks (substrate)	5	1.06	0.21	2.69 <sup>1</sup>
Error	10	0.78	0.078	

<sup>1</sup>not significant

\*significant ( $P < .05$ )

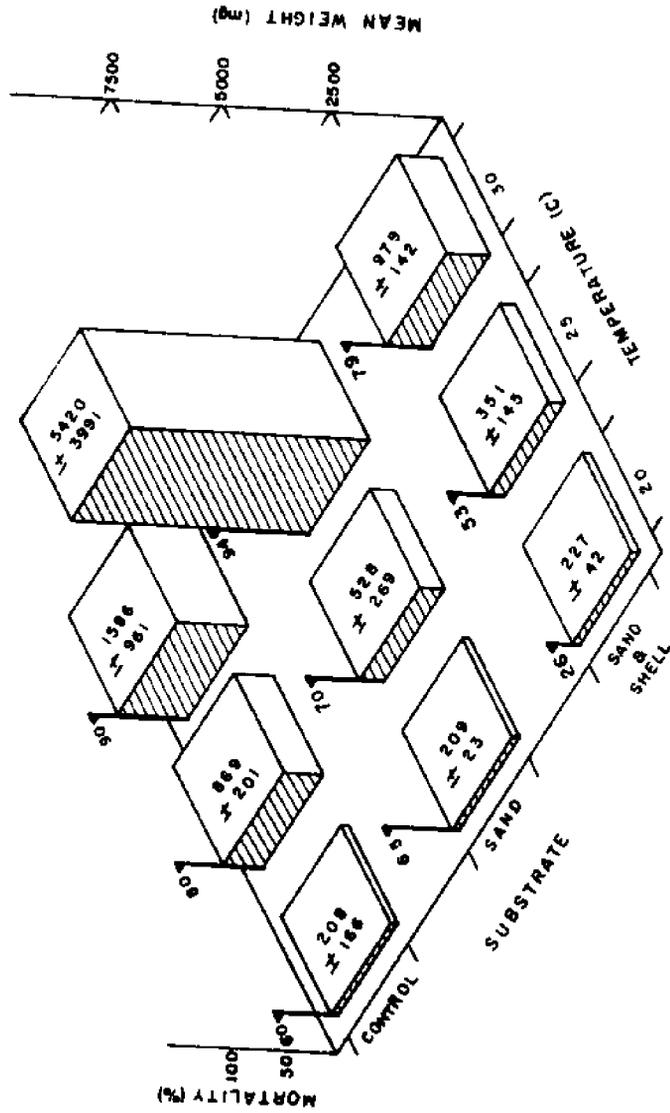


Figure 32. Mean yield of juvenile blue crab groups at nine combinations of temperature and substrate. Numbers on histograms indicate yield (weight), mean and range for replicate groups of 50 crabs. Flags on histograms indicate percent mortality.

TABLE 25.--t-tests of yields (log-transformed) of groups of uncaged juvenile blue crabs maintained at three temperatures (20, 25, and 30 C).

	Log of Yield		
	20 C	25 C	30 C
	2.57	3.02	3.40
	2.40	2.90	3.97
	2.42	2.69	2.92
	1.62	2.82	2.79
	2.31	2.4	3.15
	2.26	2.31	3.04
$\bar{x}$	2.26	2.69	3.21
t tests:	<u>20 vs. 30</u>	<u>20 vs. 25</u>	<u>25 vs. 30</u>
d	-0.94	-0.42	0.52
$\bar{x}_1 - \bar{x}_2$	0.37	0.41	0.40
N	5.00	5.00	5.00
T	6.25*	-2.50 <sup>1</sup>	3.18*

<sup>1</sup>significant (P < .06)

\*significant (P < .05)

### Acclimation of Growth-Temperature Survivors

Survival time of crabs from different acclimation temperatures subjected to the same lethal temperature was directly proportional to the acclimation temperature (Figure 33). Crabs acclimated to 27 C had survival times from 9.6-180 minutes in a 40 C lethal bath. Crabs acclimated to 35 C survived from 156 to 693 minutes at the same lethal temperature.

Six soft-shelled crabs were included in this series of lethal tests (Figure 33). There were no obvious differences between their survival times and survival times of hard crabs from the same acclimation temperature. There seems to have been a trend toward lower survival times as the acclimation temperature increases. The number of soft-shelled crabs was too small to test this hypothesis statistically.

Clumping of death times into different groups was obvious in most series of lethal tests (Figure 33). Crabs acclimated at 27 C appeared to be segregated into at least three groups according to survival time. Two groups appeared to be surviving differently at 29 C. At 31 C, the grouping was not as obvious, except that a single crab died at about the same time as the less resistant group at 29 C. Two and perhaps three different groups survival times were noted at 33 and 35 C.

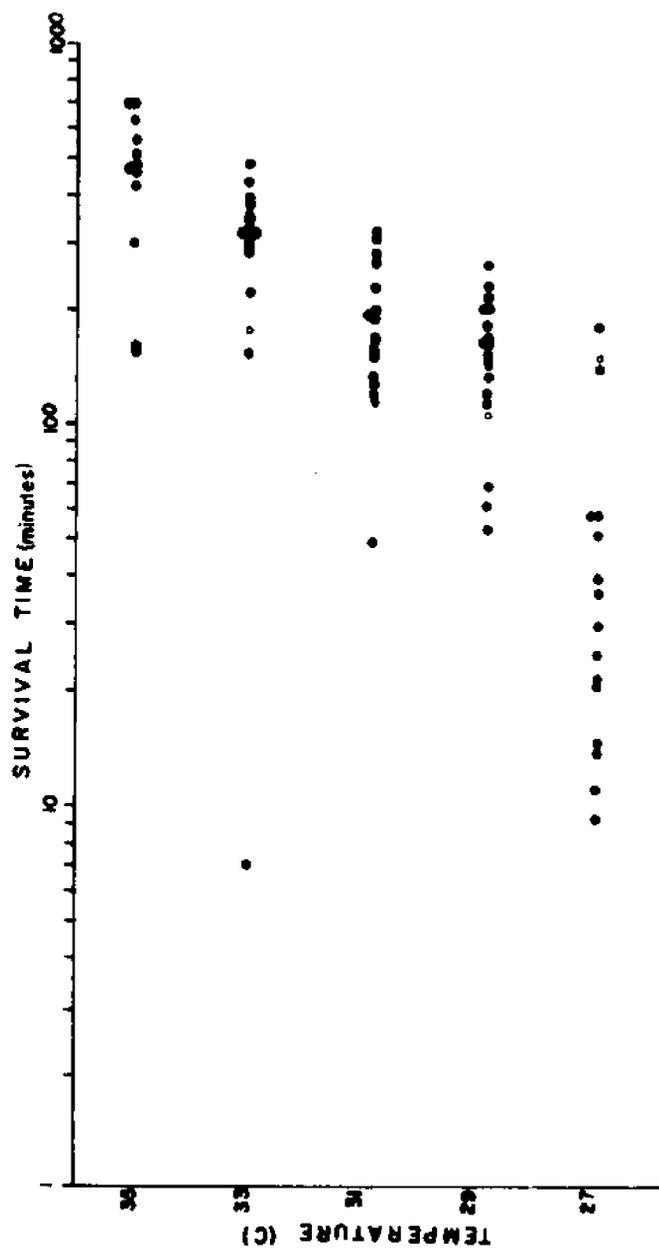


Figure 33. Individual survival times of juvenile blue crabs acclimated at various temperatures (27-35 C) and subjected to 40 C. Open circles indicate survival times of soft crabs.

There was no significant effect of sex on survival time. The t-tests used to check for differences between the means of the logarithms of survival times of males and females from each acclimation temperature indicated no significant differences.

Survival times of crabs acclimated at 27 C and subjected to 40 C lethal temperature were positively correlated ( $P < .05$ ) to both width and weight. Crabs acclimated at 29, 31, 33 and 35 C showed no significant correlation between survival time and width or weight when subjected to 40 C.

#### Thermal Acclimation of Substrate Survivors

Crabs acclimated at 20 C will apparently survive indefinitely (10,000 minutes) when subjected to 36 C (Figure 34). At 37 C, 11 of the 13 crabs tested survived less than 300 minutes. Minimum survival was 5 minutes. Survival times for crabs acclimated to 20 C shortened as the lethal temperatures increased. At 40.5 C, three of the five crabs tested survived less than 1 minute.

Crabs acclimated to 25 C, when compared to those acclimated to 20 C, showed increased survival times when subjected to similar lethal temperatures (Fig. 35). At 38.5 C, maximum survival time for crabs acclimated to 20 C was 13 minutes (Fig. 34). At the same temperature, three crabs of the 13 acclimated to 25 C tested survived

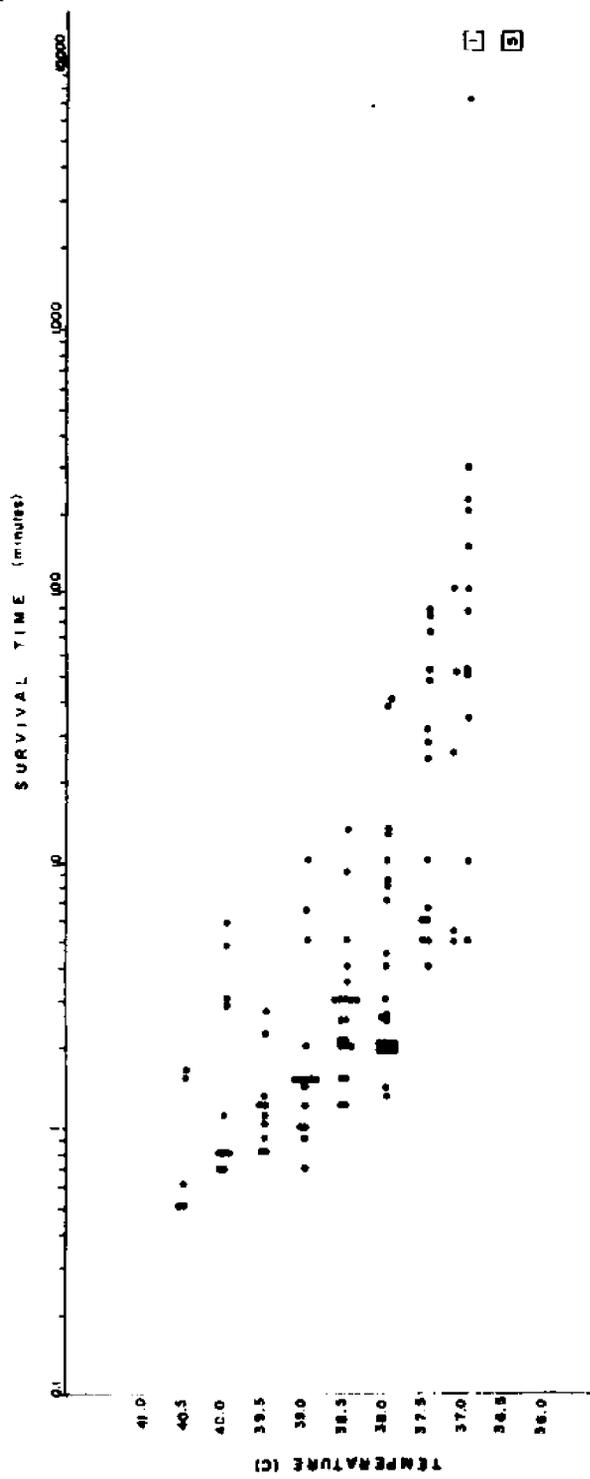


Figure 34. Individual survival times of juvenile blue crabs acclimated to 20 C for 25 days and tested at various lethal temperatures. Numbers in blocks indicate survivors after 10,000 minutes.

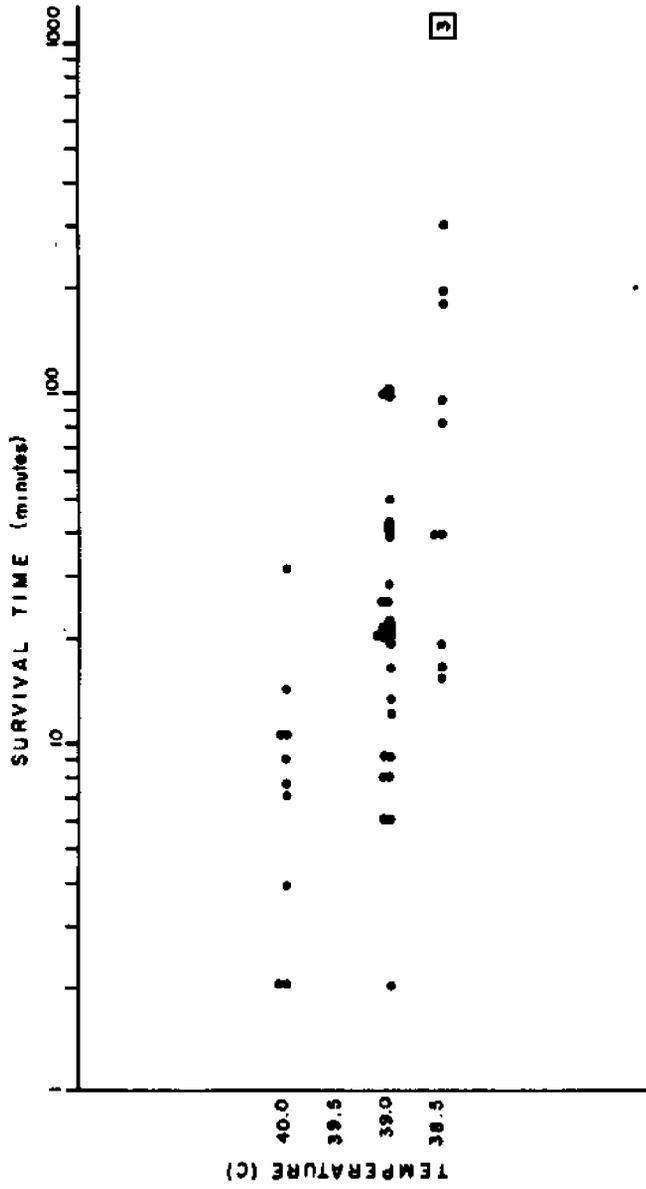


Figure 35. Individual survival times of juvenile blue crabs acclimated to 25 C for 25 days and tested at various lethal temperatures. Number in block indicates survivors after 1,000 minutes.

over 1,000 minutes with minimum survival from this sample being 15 minutes.

At 40 C, crabs acclimated to 25 C again survived longer than those acclimated to 20 C, but the difference between the two groups was not as great as at 38.5 C.

Crabs acclimated to 30 C showed increased resistance to thermal death (Figure 36). These crabs survived between 100 and 200 minutes at 40 C while those acclimated to 20 and 25 C survived less than 10 and 35 minutes, respectively.

Crabs acclimated to each temperature showed two and possibly three segregated groups of survival times at all lethal temperatures. This clumping of survival times was more diffuse at the lower lethal temperatures.

One hundred and one crabs from the substrate study lost no appendages. They were used in tests to determine effects of sex, width and weight upon the survival times of crabs from each acclimation temperature.

Since the crabs from one acclimation temperature had been subjected to a variety of lethal temperature, t-tests were run to test for possible differences in the lethal temperatures to which males and females were subjected. No significant differences between the lethal temperatures for males and females at any of the acclimation

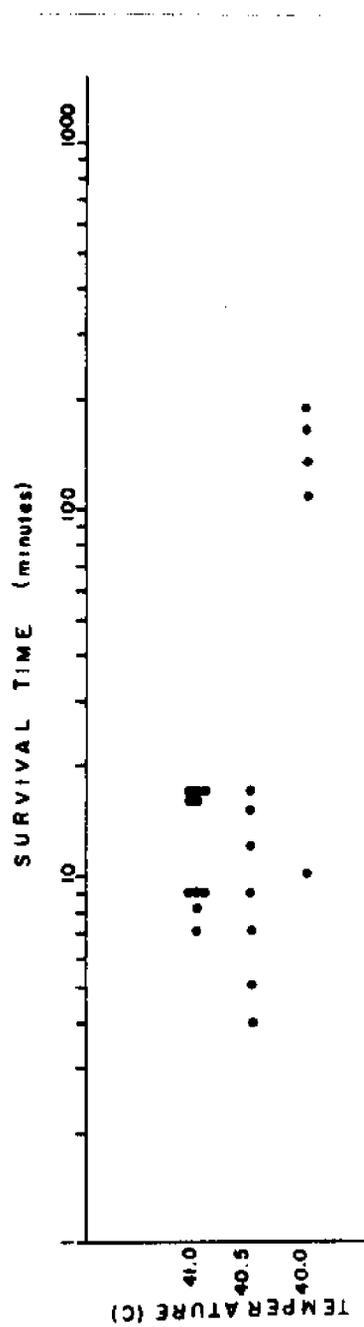


Figure 36. Individual survival times of juvenile blue crabs acclimated to 30 C for 25 days and tested at various lethal temperatures.

temperature groups were found. Similar tests were run on the survival times of males and females from each acclimation group. No differences in survival time attributable to sex were found.

There was no significant correlation ( $P < .05$ ) between width or weight of juvenile blue crabs and survival time in any group from any acclimation temperature (20, 25 or 30 C).

Body and weight ratios. --The wet weights of 101 crabs from the substrate-mortality study were 10.23 (Standard Deviation 2.6) times heavier than the underwater weights. The mean weight of this sample of crabs was 0.7269 grams (S.D. 0.7532). The wet weight to underwater weight ratio of 80 crabs from the growth-temperature study was 11.36 (S.D. 2.8695). This sample of crabs had a mean wet weight of 2.5 grams.

The substrate survivor sample had a 2.570:1 ratio of dry weight to underwater weight. The wet weight to dry weight ratio of this group was 4.131:1 (S.D. 0.976). Width to wet weight ratios were calculated for the sample of 80 crabs from the growth-temperature study. The mean width of this sample was 1.4287 times the weight (grams). Mean width to length ratio for this sample was 1.84:1 (S.D. 0.1095).

## DISCUSSION

### Survival

Commercial culture of organisms demands profitable survival levels of stocked animals. At the present time, the inability to obtain profitable survival of crabs is one of the major problems impeding blue crab culture. Survival of caged juvenile blue crabs in laboratory culture was affected by temperature and salinity. Mortality of uncaged juvenile blue crabs under laboratory conditions was due primarily to cannibalism which was affected by temperature and substrate.

Data on the effects of environmental factors on small juvenile blue crabs was not found in the literature. Studies of the effects of temperature and salinity on larval blue crabs and other crab species (Costlow and Cookhout) and on large juveniles and adults (Tagatz, 1969; Mahood et al., 1970) provided useful comparisons to the present study.

Costlow and Bookhout (1959) indicated that extremely small percentages of blue crab larvae survived to the first crab stage even at temperatures and salinities which were considered optimal. They report that the major mortality occurs in the zoeal stages. The survival of zoeae from different egg masses was highly variable.

Costlow and Bookhout (1967) found that upon reaching the megalops stage, survival and rate of development were consistent among the different egg masses.

Temperature effects.--Individually-caged juvenile crabs showed varying rates of survival in a temperature range of 27-35 C. The lower temperatures (to 30 C) produced minimal mortality. Above 30 C the survival rate decreased rapidly, with only 20% survival at 35 C after 45 days.

It is possible that the increase in mortality at higher temperatures was a result of starvation. Each crab was fed the same amount daily regardless of size or acclimation temperature. An increase in metabolic activity at higher temperatures on a given amount of food could have resulted in starvation.

One sample of crabs used in a lethal test to be discussed below survived 10,000 minutes (7 days) at 36 C with no feeding. There is a possibility that this sample of crabs maintained a lower metabolic rate for part of the 7 days due to its acclimation at 20 C. Vernberg and Vernberg (1966) showed that muscle tissue from cold-acclimated fiddler crabs, Uca pugnax, showed lower respiration rate than did muscle tissue from warm-acclimated individuals when they were subjected to the same temperature range (10-15 C).

Tagatz (1969) reports acclimating juvenile (40-60 mm) and adult blue crabs at several temperatures, including 30 C, for 21 days and feeding them only once. I fed crabs daily at a feeding rate thought to have been high enough to have countered the metabolic rate increase due to a 5 C increase. In the temperature-growth (T-G) experiment, crabs were fed 66% of the initial mean weight, which was 10% of the final mean weight daily.

Mortalities were replaced in this experiment and several of the replacement crabs died within several days after being introduced into the 31-35 C range. I do not believe that starvation played a major role in the increase of mortality of juvenile blue crabs at higher (31-35 C) acclimation temperatures in this study.

Several possible explanations for the increase in mortalities at elevated temperatures exist. There is a possibility of disease affecting survival at the higher temperatures. The unusually high rate of mortality in both replicate groups of crabs maintained at 31 C may be indicative of disease. No gross evidences of disease were observed. The mortalities occurred throughout the 45-day study period with no apparent distribution which might indicate disease.

A second possibility is that temperatures above 30 C are in the zone of resistance of juvenile blue crabs. Fry (1956) defined the zone of resistance as the temperature regime above and below the

temperature range compatible to the continued existence of the organism. The zone of resistance is delineated by the upper and lower incipient lethal temperatures which are defined (Fry, 1956) as the temperature levels at which 50% of the sample survives an exposure, indefinite in its duration, longer than any period of time sufficient to kill half the sample at any temperature which is lethal. According to this definition, the upper incipient lethal temperature for juvenile blue crabs based on mortality in 45 days in 33 C at 15‰ salinity. At 15 ‰, the lowest temperature at which 50% of the crabs died in 45 days was 33 C.

The possibility that thermal stress caused increased mortality at temperatures between 31-35 C is increased by the indication that crabs in nature may not often be subjected to temperatures in this range. Monthly mean temperatures in Texas estuaries rarely exceed 30-31 C with 35.5 C as an extreme diurnal value reported by Truesdale (1970) for a collection station in the Upper Trinity Bay.

It may be that juvenile crabs can stand temperatures in the 31-35 C range indefinitely under natural conditions where water temperatures fluctuate through a diel period. Further laboratory research including testing of survival of small crabs at variable temperature patterns in this range is needed.

Mortality of blue crabs due to low temperatures in nature has been reported (Moore, 1969; Tagatz, 1969). Mahood et al. (1970) reported no survival of adult blue crabs in any salinity at 0 C after 96 hours. Survival of adult blue crabs increased with temperature, with optimal survival temperatures influenced by salinity. Low salinities were beneficial to survival at low temperatures but detrimental at high temperatures. High salinities had the opposite effect, being beneficial at high temperatures and detrimental at low temperatures (Mahood et al., 1970).

In the present study, an experiment comparing growth rates of small blue crabs at 29 and 15 C in four salinities (1, 2, 4 and 6‰) indicated that 15 C may produce mortality of small blue crabs. Mortalities did not occur at 15 C until the 24th day of the experiment. The small crabs began to die in increasing numbers until the experiment was terminated 6 days later. This mortality was thought to be due to exposure to the low temperature. No gross evidence of disease or poor water quality was seen. The crabs at 15 C did not eat or grow well. A few of these crabs molted early in the experiment but the molt rate tended to increase in the latter part of the experiment. The stress of molting is thought to have contributed to the mortality of small blue crabs after a long exposure to 15 C.

These crabs were caged and unable to bury themselves in a particular substrate. More (1969) reports that adult male blue crabs bury in mud at 15 C. It is probable that few juvenile blue crabs are killed in natural populations by 15 C. Escape mechanisms such as burrowing and movement to more suitable temperatures are probably exercised by small blue crabs in natural populations when temperature extremes are encountered.

The comparison of growth and mortality of small blue crabs at 15 and 29 C was terminated after 30 days. Mortality in the warmer temperature was limited to 1‰ salinity while it was observed (after 24 days) at all salinities in the cold water. I feel that had the study been continued, much more mortality would have been observed at the lower temperature while that at 29 C would have remained limited to crabs at 1‰ salinity. Thus, a temperature effect on mortality might have been observed since the mortality at 15 C had just begun and had already surpassed that at 29 C. However, at the termination of the experiment there was no significant difference between 15 and 29 C in producing mortality (Table 11).

Survival of groups of 50 uncaged juvenile blue crabs was significantly different between temperatures (Table 17, Figure 28). Survival decreased as temperature increased. Mortality in this experiment was not directly attributed to temperature but to

cannibalism. At the higher temperatures, small blue crabs were more active and had increased metabolic rates. Low feeding rate also probably contributed to mortality. The more active crabs were more prone to cannibalism. A second effect of temperature on mortality involved growth. The crabs at higher temperatures tended to grow faster and molt more often. Consequently, they were susceptible to cannibalism more often.

Salinity effects.--The apparent lethality of 1‰ to small juvenile blue crabs has not been previously reported. Mortality induced by low salinities has been reported for larval blue crabs and other crab species. Costlow (1967) reported 100% mortality of blue crab megalops at 5‰ at 20 C. Costlow, Bookhout and Monroe (1966) found no survival of zoeae of Rhithropanopeus harrissi in 1‰ salinity at 20, 25 or 30 C. Survival for these zoeae was found to increase as salinity ranged from 2.5‰ to 25‰ and then to decrease as salinity ranged from 25‰-40‰ at 20 C.

Zein-Eldin and Aldrich (1965) reported similar temperature-salinity interactions for postlarval penaeid shrimp. They observed no mortality at temperatures of 20-32 C in waters of 5-37‰ salinity. Temperature became critical at salinities less than 5‰.

The inability to cope with extremely low salinities for larval forms, such as those of C. *sapidus*, which are spawned in full marine salinities is undoubtedly due to genetic adaptation for high salinities. In species such as the blue crab, which spends much of its postlarval life in estuarine areas, the ability to tolerate low salinities must be gained after the larval stages.

Small juvenile blue crabs have been reported by many authors as actively moving to areas of low salinity (Gunter, 1950; Darnell, 1959; More, 1969). More (op. cit.) found that the mean catch of small blue crabs per trawl tow was greatest at a salinity range of 0.0-1.9‰ in Texas estuaries. Truesdale (1970) reported finding juvenile blue crabs (<20 mm) in low salinities ( $\leq 1‰$ ) in the lower Trinity River and upper Trinity Bay area.

Mortality observed at 1‰ salinity in this study was strongly related to molting. The obvious swelling and distention of the dead crabs appears to be due to the intake of too much water. Crustaceans swallow and absorb large quantities of water to increase their size during ecdysis (Passano, 1960). When water is swallowed, the hydrostatic pressure in the gut is increased. The combined effects of filtration and osmotic uptake cause the water to pass into the hemolymph. Blood volume may double, causing the animal to increase in size. In the case of juvenile blue crabs at 1‰, too much

water is obviously taken in. This may be due to the inability of the small crabs to control the osmotic process. The control seems to be lacking in the larval forms and is reported (Tan and Van Engel, 1966) to be differentially developed in adult males and females. Mahood et al. (1970) reported that adult blue crabs subjected to lethally high temperatures survived less well at low salinities than in high salinities. They cite work by King (1965) showing that low salinities had a marked increase on blue crab metabolism. They concluded that high temperature and low salinity produced a combination of increased metabolic and osmoregulatory stress and thereby decreased the upper  $TL_m$  at such combinations.

If juvenile blue crabs cannot cope with salinities of 1‰, how can their well-documented migration into low salinity water be explained? Small blue crabs may not move far enough into estuaries to reach 1‰ salinity under normal conditions until their osmoregulatory capabilities have developed. Since mortality in low salinity (1‰) occurs only at molting, a sudden freshet of moderate duration would produce little mortality. It has been observed (Passano, 1960) that blue crabs can retard and initiate molting relative to tidal cycles. This ability may also be extended in small blue crabs to salinity conditions. Some crabs did survive at least one molt at 1‰ salinity, but all mortality in this salinity was associated with molting.

The temperature-salinity interaction observed affecting mortality of small blue crabs may be involved in accounting for their known distribution in light of the lethality of 1‰ salinity. At warm temperatures (29 C), extremely low (1‰) salinity levels were shown to be lethal, while at low temperatures (15 C), there were no significant differences in mortality due to salinity. During the warm summer and fall months in Texas, slight rainfall and increased evaporation rate would lessen the probability of extremely low salinities. During cooler months, mortality due to low salinity would be minimized both by less molting and by the fact that 1‰ does not appear to be lethal at low temperatures. More's (1969) finding of small crabs in 0.0 to 1.9‰ salinity may be due to his lumping data from several years' collections and thus finding small crabs at low salinities when temperatures are not high enough to provide the lethal interaction. Pearson (1948) reported that over a 15-year period, high discharge from the James River during May and June was correlated with low crab survival. He attributes this to lowering of the salinities of the crab spawning areas. Another possibility would be that small crabs over-wintering in the area were killed by the low salinity during high spring runoff which significantly affected the crab population. He found low river runoff to be correlated with high crab survival.

Judy (1966) reported that the catch-per-unit of effort of small blue crabs (juveniles) in the Core Sound, N. C., area was highest in March during both years of a 2-year study. Catch-per-unit effort sharply increased as water temperature increased in the sound. He attributed the increased catch to movement of the small crabs from creeks to more open (saline) water. Further study (Judy, 1967) indicated that fluctuation in catch of crabs in the low salinity water was related to crab size. Small crabs (< 1-1.5 inches) were abundant in freshwater during the winter and spring (November-April), practically none were in the freshwater during the summer months. Older juveniles showed some fluctuation and no similar fluctuation was seen for adult crabs. Crabs in the present study were in the small size range reported by Judy. Truesdale (1970) reported very similar fluctuations in small (< 20 mm) blue crabs in the upper Trinity Bay. He attributes the peaks during the winter months to recruitment, but this data may also indicate the migration of small blue crabs to avoid the high temperature-low salinity combination.

Gallaway (1970) in sampling blue crab abundance at the discharge area of the P. H. Robinson Generating Station on Galveston Bay, reported high abundance of crabs at most trawl stations. The one time no crabs were caught at these stations coincided with 27-30 C temperature and low salinity (0.4-2‰).

Salinities above 1‰ (2-2 1‰) had no effect on mortality of juvenile blue crabs. Since blue crabs are primarily estuarine organisms, particularly as juveniles, euryhalinity is an important adaptive feature.

Substrate effects.--Mortality of crabs due to cannibalism has been widely documented (Iversen, 1968; Ryther and Bardach, 1968; Costlow, 1967). In the present work, substrates which provided different amounts of cover varied significantly in reducing losses to cannibalism. A substrate of coarse sand provided no increase in protection over a glass bottom. Small crabs were seen to partially bury themselves in the sand which would seem to provide some cover. It is probable that the majority of mortalities from cannibalism occurred when the crabs were soft following ecdysis. Soft crabs would be unable to burrow into the sand substrate and would be unprotected during their most vulnerable period. It appeared that cover, in the form of oyster shells such as used in this experiment or similar forms of cover in nature, are required to minimize mortality from cannibalism. The significance of this need is readily seen in the natural distribution of juvenile blue crabs. I found them in greatest numbers generally in shallow water areas with soft bottoms and available cover.

The mortality rate attributed to cannibalism in the substrate study did not vary with time. Fifty juvenile blue crabs were stocked in each of the 7.5-gallon aquaria. As the test population in each aquarium was diminished in number through time, a corresponding lessening of mortality due to cannibalism was expected but not observed. Crabs became larger as populations became smaller. The study was terminated when one test population was comprised of one large crab.

It is possible that the sand-plus-shell substrate would have produced a leveling off of the mortality rate in time. Only 25 oyster shell valves were placed in each of the sand-plus-shell aquaria. Some of those possibly did not provide proper cover. If the test population had been reduced to the point at which there was proper cover for each crab, perhaps the leveling off of mortality rate would have been observed.

Other possibilities exist, however. The level of feeding in this study was thought to be inadequate, particularly in terms of the number of individual food pellets. As the population decreased, the amount of food should have become adequate in terms of each crab getting a pellet of food at each feeding. The continuance of cannibalism may indicate that the food was not adequate in providing all the nutritional needs of the small crabs, or that small blue crabs

prefer other small blue crabs to artificial food. The latter is a distinct possibility.

### Food and Feeding

Food and rates of feeding are of primary concern in commercial culture of any organism. In established cultures of fishes, the utility of a commercially-produced, artificial feed has been recognized (Lewis et al., 1969; Locke and Linscott, 1969). This type of food provides consistent nourishment, ready availability, less water fouling and more economy than most types of natural foods for fish. Several major drawbacks to the use of artificial feeds have been recorded (Karim and Aldrich, 1970; Ryther and Bardach, 1968; Ray and Wirtanen, 1969). The acceptability of such feeds, their nutritive value, and their effects upon resistance to environmental factors have been discussed.

The blue crab is an opportunist in its feeding habits. In nature, the blue crab appears to be a predator, scavenger and detritus-feeder. It may eat anything edible that it comes in contact with. Tagatz (1968a) gave a summary of foods eaten in Florida by blue crabs of different sizes. Darnell (1959) describes the feeding practices of blue crabs in Lake Pontchartrain, Louisiana. No record of blue crabs being fed artificial, pelleted feeds was found in the literature.

The pelleted feed used in this study is not thought to be the ultimate food in nutritive value for blue crabs. It was a mixture similar to that being fed to large juvenile penaeid shrimp by George Griffith (personal communication) at the National Marine Fisheries Service Biological Laboratory in Galveston, Texas. It was composed of 50% fish flour and 50% Milk Nutrients Concentrated (MNC), a whey-like concentrate of non-fat milk nutrients. The fish flour provided a high level of protein (undetermined percentage) to the food. MNC is a livestock feed additive containing 11.5% protein, lactose, and many vitamins and minerals. It proved to be an excellent binder for ease in pellet making.

The major importance of the feed in this study was that small blue crabs ate it and apparently grew fairly well for 45 days. More research will be necessary to compound an artificial feed to meet the presently unknown nutritional requirements of blue crabs.

Juvenile blue crabs were fed at varying rates in the different experiments. Generally, I feel that crabs in all the caged-growth studies were amply fed or over-fed in terms of percent body weight fed each day. Utilization of food would probably have been better if smaller pellets had been fed more often during the day.

The crabs did not utilize all the feed fed. Much of the time a fine ooze of food particles could be found under the cages after

feeding. This was due to the maceration of the feed pellets by the crab, causing some particles to be lost and to the inability of the small crabs to eat all of the feed pellet before it softened and dispersed.

The uncaged crabs in the substrate study were under-fed. This may not have been true in terms of percent of body weight fed daily for each group of crabs but appeared so in number of pellets available. Only 10 pellets were fed daily to each group of crabs. These pellets were hoarded by those crabs that got them until they were eaten or disintegrated. Some feed was wasted by feeding 10 pellets daily to groups decimated by cannibalism in which fewer than 10 crabs survived as some of the pellets disintegrated before they could be eaten.

There were no observed draw-backs to the pelleted feed used other than rapid disintegration time. As previously noted, the small crabs took the pellets readily. Karim and Aldrich (1970) noted that some commercial feeds most readily accepted by postlarval penaeid shrimp seemed to lower the thermal resistance of the shrimp. All crabs subjected to lethal tests in this study were fed the same feed, so no comparison as to the effect of diet upon thermal resistance was investigated.

### Growth, Yield and Food Conversion

The major factor affecting growth, yield and food conversion of juvenile blue crabs during this study was temperature. Costlow (1962, 1966) noted that development of blue crab megalops at any one temperature was slower at lower salinities but the differences were less than those due to temperature. He said that temperature is the most important factor in the length of the megalops stage.

Temperature effects. --Maximum growth of juvenile blue crabs was obtained at 29-30 C in my study. Costlow (1967a) found that the megalops of the blue crabs metamorphose to the first crab stage most rapidly at 30 C. His findings indicate that the time to metamorphosis for the blue crab megalops approximately doubles with a 10 C decrease in temperature. Studies of larval development of the crabs Panopeus herbsti, Sesarma cinereum and Rhithropanopeus harrisi indicated that time to reach the different stages (zoeal, megalops and first crab) was reduced as temperature increased (Costlow and Bookhout, 1967; Costlow, Bookhout and Monroe, 1966). The highest temperature used in the larval crab studies of Costlow et al. (1966, 1967) was 30 C.

In my experiments, temperatures above 30 C generally permitted less growth of juvenile blue crabs as the temperature increased (31-35 C). Growth (measured as yield) decreased with decreasing

temperature in the uncaged samples in the substrate study (20, 25 and 30 C). The two temperatures below 29 C (27 and 28 C) in the temperature-growth study showed decreasing growth. Practically no growth was attained by juvenile blue crabs in 15 C water. It appears that growth increases with increasing temperature until an optimum temperature (29-30 C) is reached, then decreases as temperature increases to 35 C.

The metabolic rate of crustaceans is generally directly related to temperature. Wolvekamp and Waterman (1960) report that some crustaceans are able to adjust their metabolic rate to function optimally at very low temperatures. This is apparently not the case with small blue crabs. Crabs in cold water (15 and 20 C) ate very little, showed lowered activity and grew very little, presumably in response to lowered metabolic rate.

No difference was noted in the activity rates of crabs at optimal (29-30 C) temperatures and those at higher temperatures. The crabs were caged so that hyperactivity, if it occurred at elevated temperatures, was not as noticeable as the lack of activity at very low temperatures.

Temperature affected yield from groups of small blue crabs by its effect on growth and mortality. Yield in this study was defined as

the final weight of the survivors of each test population minus the weight of the original stocked group.

The effect of temperature on yield of caged and uncaged crabs was different. The general effect of yield increasing with temperature was seen for both caged and uncaged crabs but yield is also influenced by mortality. In caged crabs, yield was inversely related to mortality, so that yield above 30 C was severely affected by mortality due to temperature. The temperatures (20-30 C) in the uncaged crab study were not lethal to the crabs but did affect mortality due to cannibalism. Yield was directly related to mortality from cannibalism, which increased with temperature. The result was that with caged crabs, yield increases with temperature (to 30 C) due to increasing growth but was depressed somewhat by increasing mortality above 30 C. Yield of uncaged crabs increased with temperature due to increased growth but was also increased as explained below due to the increasing cannibalism-related mortality.

The effect of cannibalism upon yield may be due to several factors. The survivors in the test populations, in which cannibalism was high, were probably the largest crabs in the original group. They had several advantages in that they could command more than their share of the available food, could have the best cover and probably had a slightly longer time between molts than smaller crabs. As they

grew and molted, their size alone may have intimidated the smaller crabs, protecting them while they were soft. The larger crabs then were more able to effectively utilize the foods available to them, both artificial and live (smaller blue crabs). Growth of crustaceans follows an exponential curve. The small crabs were at the lag phase, growing slowly at an increasing rate. Those crabs that were able to get all the available food moved up into the logarithmic phase of the growth curve. This enabled relatively few crabs to produce much more yield than many small crabs. In one test population the one surviving crab weighed more than the survivors from five other test populations at that temperature (30 C) combined.

Other possibilities exist explaining the direct relationship between yield and mortality in uncaged crabs. It may be that increased growth due to the higher temperatures more than compensates for the losses due to cannibalism. Comparable growth of crabs in caged studies was not seen at comparable temperatures, however. The increased yield of groups with greater mortality may indicate that food was limiting growth in this experiment.

The effect of cannibalism on yield was primarily responsible for the lack of significant effect of substrate on yield (Table 24). More crabs survived in the sand-plus-shell substrate groups at all temperatures (Figure 28) but because the fewer crabs surviving in the other

substrate types produced greater yield per individual, no significant difference in yield due to substrate was observed.

Food conversion efficiency was defined as the decimal fraction indicating the relationship between the amount of weight gained and the amount of food fed. Thus, food conversion efficiency was dependent upon growth, which was temperature related. Generally food conversion efficiency varied directly with temperature to 30 C and then inversely with temperature from 31-35 C.

Food conversion efficiencies were calculated, based on the assumption that all food was consumed. This is known to be in error. Much of the food was wasted in all studies, so the food conversion efficiencies are probably too low in most cases. The amount wasted appeared to be relative to crab size. Smaller crabs wasted more food than large crabs probably because of the inability of the small crabs to eat the whole pellet before it disintegrated. With smaller pellets and multiple feedings each day the food conversion efficiencies of all crab sizes could be increased.

The maximum food conversion efficiency for 45 days was 0.27, indicating that the crabs in this group (29 C; 15‰ salinity) produced about 1 gram of weight for each 4 grams fed. This is fairly low when compared to catfish which may have food conversion ratios of 1.5:1

or better. The catfish food conversion ratios are generally calculated for the time from stocking of fingerling catfish until harvestable size.

Salinity effects. --Salinities, other than 1‰, within the range used in this study (1-21‰) had little effect on growth, yield or food conversion efficiency. One part per thousand salinity, as previously discussed, was apparently lethal to small blue crabs at 29 C (Table 11). It also hindered the growth (yield) of small crabs in the same temperature (Table 14, Figure 23). At 15 C crabs grew very little at any salinity and 1‰ apparently was not significantly different in growth production than any other test salinity at low temperature.

Since blue crabs, particularly small sizes, are most often found in estuarine waters of varying salinity, the ability to grow equally well in a wide range of salinities is an important adaptation for this organism.

There are reports of very large blue crabs found in extremely low salinity (fresh) water (More, 1969; Haefner and Shuster, 1964). A hypothesis explaining this phenomena in terms of slightly greater increases at each molt due to greater water intake by osmosis in freshwater has been proposed (Porter, 1955, and Cargo, 1958, cited by Haefner and Shuster, 1964). Haefner and Shuster (op. cit.) report no significant differences in the length increment of female blue

crabs during the terminal molt when held in varying salinities. There was no indication of greater growth (weight measurement) of juvenile blue crabs in different salinities in the low salinity (TSG) study, when 1‰ salinity is not considered (Figure 24). Salinities (6, 11, 16 and 21‰) were compared as to growth production at 29 C in the salinity growth study (Figure 18). No significant differences were indicated either after 20 or 45 days in growth of juvenile blue crabs at these salinities. However, by comparing mean growth after 30 days of groups of crabs from 2, 4 and 6‰ at 29 C (Figure 24) with mean growth of groups of crabs at 6, 11, 16 and 21‰ at 29 C after 30 days (Figure 18), a general trend of increased growth at the lower salinities was noted. This is not to be interpreted as support for the theory of greater crab growth at low salinities. As previously noted, water quality in the salinity-growth study was suspect. A change of water in all experimental tanks between the 18th and 22nd day of that study was followed by an upsurge in the mortality rate. Thus, the poor growth noted in the higher salinities (16 and 21‰) in Figure 18 may be due to the fact that more of the suspect seawater was required to mix the higher test salinities. This argument is enhanced by noting (Figure 18) that the growth rate of groups of juvenile blue crabs in 11 and 21‰ salinities were very similar for the first 20 days, with those crabs in 21‰ salinity dropping off drastically after 20 days.

The same response is noted between crab groups in 6 and 16‰ salinities. The initial weights of the groups at these salinities were very similar as was the rate of growth until 20 days. The growth rate of the group in 16‰ salinity fell far behind that of the 6‰ salinity group after the addition of the suspect seawater. Crabs maintained at 29 C and 15‰ salinity in the temperature-growth study had a higher mean weight after 30 days (Figure 9) than did those crabs in the lower salinities (Figure 24). The initial total weight of all groups of crabs used in comparison of growth at 29 C and different salinities was very similar. The statistical non-significance of salinity in producing growth of yield (other than at 1‰) and the fact that groups of crabs in 15‰ salinity (Figure 9) at 29 C grew better than did groups at lower salinities plus the probability of bad water quality causing poor growth in the higher salinities (Figure 18), indicate the salinity, other than 1‰, had little effect on growth and consequently, on yield or food conversion efficiency. Since yield is affected by mortality as well as growth, salinity at 1‰ did affect yield not only through its detrimental effect on growth, but also due to the increased mortality of 1‰ (29 C).

Substrate effects. --The effect of substrate on growth, yield and food conversion has been previously alluded to. Graphically

(Figure 37) and statistically (Table 24), there appears to be no significant effect of substrate on yield as a measure of growth. Different substrates did produce significant results in survival of juvenile blue crabs. The direct relationship between cannibalism and mortality observed probably concealed the real effects of substrate on growth (yield) of juvenile blue crabs. I feel that yield in weight of post-larval (early juvenile) blue crabs is of little importance to prospective culturists of blue crabs. The real importance lies in survival of the maximum number of juveniles to sub-adult stage. Thus, the non-significance of substrate in yield production has little practical significance.

### Molting

Molting of juvenile blue crabs is a function of growth and as such should be affected by environmental parameters affecting growth. Molting was generally observed in this study by observation of the exuviae while feeding the small crabs.

In some parts of this study the crabs were fed only once per day. At most crabs were fed twice daily. Several times crabs were observed to eat the cast exoskeleton between feedings. It is thus felt that many molts were probably unrecorded, particularly in those experiments in which the crabs were fed once daily.

Those molts that were observed were recorded daily. Upon tabulation of the number of molts observed in groups from different temperatures and salinities, no significant differences were observed. This was thought to be due to the fact that much data was missing. The intermolt period for all crabs that molted more than once were calculated (Figure 15). It is apparent from this figure that the period between molts decreased with optimum growth temperatures (29-30 C) and increased at temperatures above and below optimum growth temperatures. Thus, more molts should have occurred at the optimum temperatures but were not recorded as such.

The intermolt period appears to be around 6-9 days at 29-30 C. Maximum time between molts appears to be 11-14 days at 27 C.

One aspect of molting which could not be considered in this data was the effect of size on molting. Too few molts were recorded for each crab to indicate any differences in period between molts due to increasing size.

Further corroboration of the non-significance of salinity in affecting growth of juvenile blue crabs was seen in the lack of effect of salinity upon the period between molts (Figure 20). There was clearly little difference in the mean period between molts due to salinity. The mean intermolt period appeared slightly longer than that observed in the temperature-growth study at 29 C.

### Thermal Acclimation

Rate of thermal acclimation, upper thermal-tolerance limits and the effects of acclimation temperature on thermal tolerance of juvenile blue crabs are of major importance to prospective crab culture. Thermal tolerance of juvenile blue crabs may also be important in interpreting their distribution.

The rate of thermal acclimation of small blue crabs as determined by this study is much more rapid than previously reported for blue crabs and is similar to rates reported for other crustaceans. Juvenile blue crabs were collected at 27 C and acclimated to 35 C. No difference was observed in the survival time of crabs acclimated for 4 and 8 days, suggesting completion of acclimation to 35 C in 96 hours. This finding is somewhat weakened in that the number of crabs available for the 8-day lethal test was small (2). The mean survival time for this sample corresponded very well with that of the 4-day sample.

Tagatz (1969) collected adult blue crabs at 12-22 C and acclimated them to 22 and 30 C. He reports a 21-day acclimation period. Tagatz op. cit.) cites the work of McLeese (1956) with the lobster, Homarus americanus, as indicating slow acclimation rate for another decapod crustacean. Wiesepepe and Aldrich (1970) found that post-larval brown shrimp, Penaeus aztecus, acclimate in about 3 days

with no further acclimation for 5 days. This corresponds very closely to the present findings for juvenile blue crabs. Studies of various crustaceans have indicated acclimation periods of 3 days or less (Spoor, 1955; Bowler, 1963; Sprague, 1963).

The acclimation rate of juvenile blue crabs needs to be studied for a period of at least 30 days. Further acclimation may occur after the 8-day period used in this study. However, in the rapidly changing environment of Texas estuaries, a short acclimation period would be very beneficial to small blue crabs. It is felt that the majority of acclimation of small crabs does occur in 4 days.

The upper incipient lethal temperature of juvenile blue crabs as indicated by mortality over a 45-day period in temperatures from 27-35 C appears to be 33 C as discussed previously. Survival in the zone of resistance is primarily dependent upon the acclimation temperature. One thousand minute median tolerance limits ( $TL_m$ ) were found to be 37.1, 38.6 and 39.4 for crabs acclimated to 20, 25 and 30 C, respectively. Tagatz (1969) estimated the 48-hour  $TL_m$  of large juvenile blue crabs (40-60 mm) acclimated to 22 C to be 37.0 C and 39.0 C in 20 and 100‰ seawater, respectively. Tagatz (op cit.) indicated that the 48-hour  $TL_m$  for juvenile blue crabs acclimated to 30 C was 37.2 and 39.0 C, respectively, for 20 and 100‰ seawater.

There appears to be little or no difference in the thermal tolerance of the different sized juveniles when comparing Tagatz' data to the present study. Tagatz (op. cit.) states in his conclusion that he feels that power plants are potentially harmful to juvenile blue crabs.

Seasonal abundance of small blue crabs in the effluent canal of the P. H. Robinson Generating Station on Galveston Bay showed peaks in January and July of 1968 and January, July and August of 1969 (Galloway, 1970). At the time of the summer recruitment peak of both years the bottom water temperature at the mouth of the effluent canal was 38-40 C. In light of the thermal tolerance of small blue crabs indicated by this study, I believe that juvenile blue crabs in this area were not there by choice. Several other possibilities exist, however. It may be that small blue crabs temporarily enter the heated water to feed or that they may be acclimated to higher temperatures and are able to withstand 38-40 C. Conditions in the laboratory during acclimation may decrease resistance to heat.

If the small crabs are actively entering the heated effluent to feed on the organisms killed by the high temperatures, I would expect the increases of abundance of crabs to be reflected in all collection data and to be similar for all crab sizes. Generally, Galloway's seining data (1970) does not show the increase of small crab abundance within the heated effluent that the trawl data does. The patterns

of trawl data and seine data are fairly consistent but different. This may be due to differences in gear or habitat. This pattern could also be explained by the entrainment hypothesis to be discussed below.

There was indication (Galloway, personal communication) that large blue crabs tended to be collected more often in the heated waters than at cooler stations. Numbers involved were small, so this was not as easily discernable as with the congregation of small crabs.

Another hypothesis explaining the abundance of small crabs in the hot water is that the crabs come up Galveston Bay and acclimate to temperatures above 30 C in the fringes of the effluent water mass so that they can move into 38-40 C water. This appears unlikely. Small blue crabs were acclimated to 35 C for 6 days and subjected to a series of lethal temperatures (Figure 7). The sample subjected to 39.5 C died between 300-900 minutes. The influence of tides and winds on the heated mass of effluent water tends to move it up and down the bay which would make it difficult for small crabs to stay in the fringe of the warm water to acclimate to 35 C.

It may be possible that laboratory conditions, particularly food, may lower juvenile blue crab resistance to high temperatures. As previously mentioned, Karim and Aldrich (1970) did find that some artificial foods lowered the thermal tolerance of shrimp. The present

findings of juvenile blue crab thermal tolerance were very similar to that of Tagatz (1969), but Tagatz did not record what he fed acclimating crabs. Mahood et al. (1970) found no survival at 36 C of adult blue crabs in any salinity lower than 30.1‰ and they compare their findings favorably to those of Tagatz (1969). They fed natural foods (cut bait) to acclimating crabs.

The hypothesis most acceptable to me explaining the accumulation of juvenile blue crabs in the 38-40 C water of the effluent canal mouth during July and August is one of entrainment of blue crabs.

The water temperatures in the Galveston Bay complex are quite warm during the summer. It is reasonable to assume that the small blue crabs being recruited into the area are acclimated to temperatures very close to 30 C.

Small blue crabs moving into Dickinson Bay may be entrained in the intake canal, by-pass the power plant and enter the heated waters of the effluent canal near the power plant or very small crabs may pass through the power plant. Assuming acclimation to at least 30 C, the 1000 minute  $TL_m$  of the small crabs is approximately 39.4 C. Most of the small blue crabs could survive in the effluent canal for at least 1000 minutes, depending upon temperature. They may be temperature shocked into a state of disorientation upon introduction into the effluent waters. The swift current in the effluent canal could

carry the small crabs to the mouth of the canal in a matter of hours, a trip which most would probably survive. At the mouth of the effluent canal, the effluent stream widens considerably and is guided bayward by two groins. At this point the water is somewhat cooler than near the plant and soft substrate may be more available than in the swift moving waters of the effluent canal proper. The small blue crabs, presumably no longer completely disoriented by heat shock, sensing cooler waters and less flow rate, bury themselves in the substrate to escape the still lethally hot temperatures. If there is a soft substrate in the effluent canal proper, some crabs may bury there or may be unable to do so due to initial heat shock. At the mouth of the effluent canal, the crabs may bury into the substrate, leave the area or be killed by the heat. The small blue crabs collected in the area of the mouth of the canal tend to be associated with the bottom substrate (Gallaway, personal communication).

The accumulation of small blue crabs in the mouth of the effluent canal at times of peak recruitment then may stem from a burrowing response to high temperature; to a decrease in flow rate; to increase in substrate; or to regaining of orientation after heat shock as described above and by Tagatz (1969). Those crabs that do not bury may not be able to avoid the trawl as well as crabs in cooler water.

Size class distribution of juvenile blue crabs from power plant effluent data (Gallaway, 1970) may be indicative of small crabs leaving the heated waters or being killed. Months of peak recruitment in which large numbers of small (< 20 mm) blue crabs are found in the heated waters are not followed by corresponding increases in large size classes in the following months. This was observed at all stations, not just those stations in the heated water, so that natural mortality due to predation may be acting equally at all collection stations. If this is true, the hypothesis of the hot water as a predator-proof refuge for small blue crabs is questioned.

I feel that the finding of large numbers of small blue crabs in 38-40 C water is a strong argument for inability of small organisms to avoid the unfavorable, possibly lethal, conditions of the power plant effluent.

It may be that small blue crabs seek out the warm waters during periods when ambient temperatures are low. This apparently is true for many fish species around the P. H. Robinson Generating Station effluent canal (Gallaway, 1970).

Further studies are needed to resolve the apparent differences between the findings of the lethality of 38-40 C in this study and the findings of Gallaway (1970) of concentrations of small blue crabs at these temperatures in a power plant effluent. I would suggest a

thermal tolerance study of caged juvenile blue crabs, collected from the intake canal and subjected to the effluent canal when the water temperature is 38-40 C. Ideally this test should be run over a range of salinities as low salinities have been shown to be detrimental to tolerance to lethally high temperatures for adult blue crabs (Mahood et al., 1970) and for large juveniles (Tagatz, 1969). Such a study, including crabs that could burrow into the substrate and be retrieved would be of great value in resolving the different interpretations of effects of power plants upon small blue crabs during periods of high (38-40 C) water temperatures.

Different physiological mechanisms thought to be associated with death at high temperatures of vertebrate poikilotherms include osmoregulation, lactic acid concentration in muscles, and some effect on the central nervous system (Fry, 1967). Allen and Strawn (1967) reported the presence of approximately six "lethal effects" which appear to separate channel catfish into different physiological groups when subjected to lethal temperatures. It was reported that different lethal temperatures allow the expression of different numbers of "lethal effects" on the longear sunfish, Lepomis megalotis (Neill, Strawn and Dunn, 1966).

The physiological mechanisms causing death of small blue crabs in this study are unknown. At least two and perhaps three different

mechanisms are seen as more or less clearly separated groups of survival times. The reactions of the crabs that died very quickly were thought to resemble shock and would be involved with the central nervous system.

No gross differences in the survival times of juvenile blue crabs due to size or sex were noted during the lethal tests. Statistical procedures employed (correlation coefficients and t-tests) detected no difference in survival times of males and females or of different sized crabs in this study. Tagatz (1969) postulated no differences between the survival times of blue crabs due to sex but found slight differences between the survival times of adult females and large juveniles. The differences in survival times for different sized organisms at high lethal temperatures is often due to the difference in time taken for heat to penetrate through a large body as opposed to a small one. Perhaps the small size of all the crabs in this study (5-40 mm) resulted in little variance in death time.

Very few soft crabs were subjected to lethal tests in this study. Those that were showed no gross differences in survival time from hard shelled crabs (Figure 33). No statistical procedures were used to compare survival times of soft and hard crabs due to small number of soft crabs tested. It was surprising to me that survival was not grossly hindered by the soft condition of the crab. It seems that the

rigors of ecdysis, the trauma of being handled while soft and the lack of any thermal insulation provided by the hard exoskeleton would have made the soft crabs more susceptible to heat. There is too little data in the present study to indicate clearly how the thermal resistance of this stage compares to the hard crab stage.

#### Mariculture Implications

This study has indicated that 29-30 C is an optimal growth range for juvenile blue crabs. This temperature is common during the summer months in Texas estuaries. Through proper usage of thermal effluents, this temperature range could be maintained during the periods when ambient temperatures are low. Some provision for providing cooling water inflow into culture ponds during the summer may be necessary along the Texas Coast.

I believe that proper mariculture techniques could shorten the time required for a crab to grow to marketable size. Crabs in natural populations are reported to grow to marketable size in approximately 12-18 months, depending on the environmental conditions and spawning time. This period might be shortened to 6-8 months by keeping the crabs at constant optimum temperature, salinity and food for all crab stages. Costlow and Bookhout (1967) found that they could shorten the megalops stage from 58 days to 5.9 days by altering

temperature and salinity. Growth of juvenile blue crabs in the present study was rapid with many crabs reaching 40 mm in 45 days. I feel that the growth rate was reaching the logarithmic phase for most crabs when the studies were terminated. One crab reached over 40 mm in 25 days at 30 C at the expense of 49 other crabs in the original sample.

The fact that juvenile blue crabs accepted and grew on an artificial diet is of much importance to prospective crab mariculture. Research will be necessary to define the dietary requirements of this crab in order that a nutritionally adequate artificial food may be formulated.

This study indicates that salinity within the normal range of estuarine water will have little importance to the crab culturist during the early crab stages. The combination of high temperatures and salinity of 1‰ or less must be avoided at this stage.

The fairly fast acclimation rate as suggested by this study may facilitate crab culture. This and the wide thermal range of this crab indicate that within a broad range of temperatures the blue crab culturist may have relatively few problems with thermal tolerance. Temperatures between 31-35 C may cause mortality after prolonged periods of time, 50% mortality occurring at 33 C in 45 days in the laboratory.

The major problem as indicated by this study will be in controlling cannibalism among blue crabs. Substrate conditions in which cover in a form usable by a molting crab may significantly lower mortality due to cannibalism but cover of this type could impede harvest in a pond situation.

Crab culture, if practiced with the present state of technology, would depend on intensive culture of larvae and postlarvae to sub-adult (50-60 mm) size and then stocking sub-adults in temperature-controlled ponds until marketable size was reached. To make blue crab culture approach profitable levels of yield, large numbers of small juveniles (5-15 mm) would have to be stocked. This means that the culture of larvae would have to be very efficient and inexpensive. This problem and many others such as nutrition, sexual maturation in culture conditions and probably diseases have yet to be overcome. This leads me to believe that the culture of blue crabs is only a future possibility.

#### Ecological Implications

Juvenile blue crabs, as indicated by this study, cannot survive at temperatures from 37-40 C for more than a few hours, actual survival time being dependent upon acclimation. Thermal effluents during summer months in Texas estuaries have temperatures within this

range. The late summer recruitment of small juvenile crabs spawned in the early spring has been shown to coincide with this period of extreme temperatures in thermal effluents. Thermal effluents then may have detrimental effects upon small blue crabs. It has been shown that small blue crabs are found in large numbers in the extremely hot (38-40 C) water of one thermal effluent on Galveston Bay (Gallaway, 1970) during the summer recruitment period. Whether or not these crabs are being killed by the high temperatures is unknown. The reasons for their being there still is a matter of interpretation of data. I feel that the findings of the present study shed serious doubt on the possibility of the small crabs being in the hot water by choice. All the temperature data in this study suggest that temperatures as high as found by Gallaway (1970) in the thermal effluent during the summer is lethal to small blue crabs.

At other times of the year, thermal effluents probably have few harmful effects on juvenile blue crabs and may even be beneficial in providing better growth temperatures and a plentiful supply of foods in the form of heat-killed members of other, less heat-tolerant, species.

In areas where extremely low salinities are prevalent during the cooler months, small blue crabs may leave the heated effluents when salinities become 1‰ or less, due to the combined stress of

warm water and low salinity. Judy (1966) has found that in Core Sound, N.C., small blue crabs are found in cool months in shallow, low salinity marginal waters. He indicates that these small crabs move from the less saline waters to higher salinity waters deeper in Core Sound as water temperature increases. It is possible that the interaction between warm water (29 C) and low salinity (1‰) in producing mortality as recorded in the present study is being avoided and is responsible for the crab movement reported by Judy.

## CONCLUSIONS

1. Growth of juvenile crabs in laboratory tanks was maximum at 29-30 C.
2. Salinity within the range of 2-21‰ has little effect on the growth or mortality of juvenile blue crabs.
3. Salinity at 1‰ or less was lethal to juvenile blue crabs in warm water (29 C) in the laboratory. This lethality is related to the molting of the crab.
4. Juvenile blue crabs accepted and grew on pelleted artificial feeds.
5. Survival of uncaged blue crabs was enhanced by addition of cover in the form of oyster shells. Sand alone did not prevent mortality from cannibalism.
6. Cannibalism remains one of the major impediments to crab culture.
7. Acclimation to a change of 8 C seems to require 4 days, a rate of about 2 C per day.
8. The 1000 minute median lethal temperatures for crabs acclimated to 20, 25 and 30 C were 37.1, 38.6 and 39.4 C, respectively.
9. The 45-day thermal tolerance of small blue crabs is high with the upper incipient lethal temperature being around 33 C if deaths in this study were directly attributable to temperature.

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